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LIMITED GROWTH AND ABNORMALITIES IN EXCISED CORN ROOT TIPS[1]

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(WITH SIX FIGURES)

Introduction

In earlier reports (**8, 9, 10, 11**) from this laboratory, attention has been called to the limited growth of excised corn root tips in a sterile modified Pfeffer's solution containing 2 per cent dextrose, and to the beneficial effects of the addition of peptone or of autolyzed yeast to this medium. Associated with the limited growth is the development of abnormalities in the roots. Aside from the condition of the roots at the time of their transfer to the culture flasks and the environmental conditions other than the culture medium itself, the obvious explanation for the limited growth and the development of abnormalities in the modified Pfeffer's solution may be placed in one of two categories. Either the medium is inadequate in one or more respects or it is injurious because of the presence or development of some injurious condition. In the present paper a further study of the limited growth and development of abnormalities in excised corn root tips under sterile conditions is reported.

We have investigated as possible causal factors the water used in preparing the medium, the carbohydrate used, the mineral salts, hydrogen-ion concentration, aeration, and the effect of certain additions to the medium. The experiments suggest a deficiency in the medium as the probable cause for the results.

[1] Supported in part by a Grant in Aid from the National Research Council.

Materials and methods

The methods used were essentially those reported earlier. Grains of Longfellow flint corn[2] were sterilized with calcium hypochlorite and germinated in petri dishes on a 0.5 per cent water agar. After germination, the main root tip was severed by a sharp knife and transferred to the sterile medium in which it was grown. Usually a single root tip was grown in a culture vessel.

The solutions used were a modified Pfeffer's solution previously described; a simpler and more dilute solution (solution C) prepared to imitate more closely the soil solution; and solution C to which 0.1 p.p.m. of manganese chloride, 0.1 p.p.m. of zinc chloride, and 0.1 p.p.m. of sodium borate were added. The last solution is referred to as solution CF. Solution C contained 50 p.p.m. calcium nitrate, 10 p.p.m. magnesium sulphate, 10 p.p.m. potassium dihydrogen phosphate, 1 p.p.m. ferric chloride, and 2 per cent dextrose. The dextrose unless otherwise noted was a preparation of high purity made by Coleman and Bell and labeled "Special for Injection." Culture vessels of pyrex glass were used throughout. The water employed was twice redistilled from pyrex glass.

In most of the experiments the roots were grown in 30 cc. of medium in 125 cc. Erlenmeyer flasks, and kept in the dark at room temperature without change of culture medium or transfer of the root tips to fresh medium.

Experimental results

GROWTH IN SOLUTION C.—In solution C, prepared with the Coleman and Bell dextrose, the growth of excised corn root tips was distinctly limited. The average final length of 225 tips originally 1 cm. long, in sixteen experiments performed at different times, was 8.6 cm. with an average of 39 secondary roots per root. The minimum length of a single root in these experiments was 4.0 cm. with no secondary roots, and the maximum was 44.1 cm. with 191 secondary roots. The smallest average growth in a single experiment was 5.6 cm. with an average per root of 22 secondary roots and the maximum was 15.1 cm. and 75 secondary roots.

[2] Thanks are due Dr. J. K. WILSON of Cornell University, who kindly supplied the corn used throughout these investigations.

Associated with this limited growth was the development of abnormalities, the most common of which was a water-soaked and swollen condition. In the development of this abnormality the root sinks to the bottom of the flask and the region of elongation becomes

FIG. 1.—Abnormal root after two weeks in solution C; original length 1 cm. Note swelling and the stele which is clearly visible because of water-soaked condition of root. Splitting at base is also evident.

translucent, the apical meristem standing out plainly as a whitish area due to the displacement by liquid of the gas in the intercellular spaces. The water-soaked portion, at first limited to the region of elongation, later extends backward toward the basal end of the root into the region of differentiation, and as a result the stele shows plainly through the cortex (fig. 1). Associated with the water-

soaked condition is a swelling of the cortex, increasing the diameter of the root several times in the more extreme cases and sometimes becoming so pronounced at the basal end that the cortex splits away from the central cylinder. This may occur also near the tip. Such abnormal roots grow slowly and produce branches which may not extend beyond the swollen cortex or which develop into short swollen branches or into longer secondaries with short stubby branches. The abnormal roots are stiff and friable and eventually become yellowish or brownish in color.

The first evidences of this abnormal condition may appear within a day after transfer of the roots to solution C or they may be delayed until some later stage in the growth of the root. There is considerable variation also between individual roots in the same experiment, some becoming abnormal in a day or two and others remaining normal or nearly normal for the duration of the experiment (figs. 2, 3).

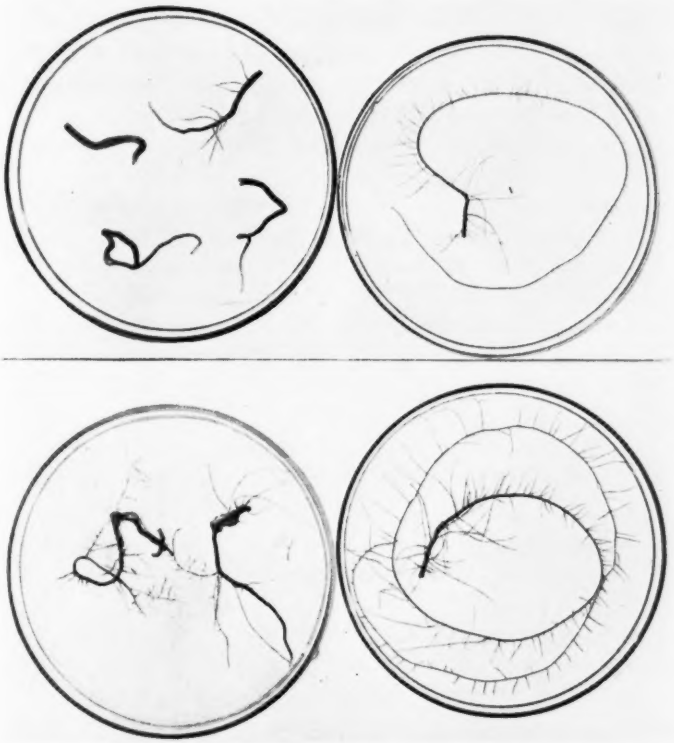
Another type of abnormality observed during the first few days of growth is a slight swelling of the region of elongation on which long root hairs had developed. The tip of the root became black and the cortex in the region of abundant root hairs swelled, wrinkled, and sometimes split away from the central cylinder. The heavy production of root hairs near the tip gave such roots a dry and woody appearance.

The proportion of roots developing such abnormalities varied from experiment to experiment. At the end of ten days or two weeks abnormal roots made up from 10 to 100 per cent of those cultured in solution C, the modified Pfeffer's solution and similar solutions.

What is the cause for these results? Is it (1) the distilled water used in preparing the medium, (2) the dextrose, (3) the mineral salts, (4) the hydrogen-ion concentration, or some other factor or combination of factors?

1. DISTILLED WATER.—It is well known that the purity of the distilled water may be an important factor in determining growth in culture solutions. In the present experiments, however, the presence of contaminants is not considered to be responsible for the limited growth and development of abnormalities in solution C. The water used was redistilled from a special still constructed of pyrex glass. The distilled water which was redistilled came from a block

tin-lined Tripure still. In some cases a small amount of barium hydroxide was added before redistillation. In other cases a trace of sulphuric acid or of sulphuric acid and potassium permanganate



FIGS. 2, 3.—Fig. 2 (above), normal root and abnormal roots from same experiment; original length 1 cm.; grown two weeks in solution C. Fig. 3 (below), normal and abnormal roots grown in solution C after pretreatment on agar or filter paper; original length 1 cm.; grown two weeks at room temperature in the dark. Left, abnormal roots (note swollen and split cortex); right, normal root.

was added before redistillation and the distillate again distilled from a barium hydroxide solution, making a triply distilled water. Triply distilled water was prepared also by distillation from acid permanganate and from a permanganate solution made alkaline with

potassium hydroxide. Little difference was found in the growth of excised corn root tips in solution C prepared with these various kinds of distilled water.

2. CARBOHYDRATE.—Next to water, dextrose (customarily used at 2 per cent concentration) comprises more of solution C than any other ingredient. It is desirable to determine, therefore, whether the kind of carbohydrate, its purity, or change in its constitution as the result of sterilization may be responsible for the results secured in solution C or similar solutions.

ROBBINS (8) reported dextrose to be superior to levulose as a carbon source for excised corn root tips grown in a modified Pfeffer's solution. WHITE (18) states that sucrose was more satisfactory than dextrose for the growth of excised tomato roots in the solution he used. The writers have compared dextrose, levulose, xylose, sucrose, and maltose as carbon sources for excised corn root tips. The results show dextrose to be somewhat superior to levulose or sucrose and very much better than either xylose or maltose.

The brands of sugars used in making the comparisons were: dextrose, anhydrous (Eastman Kodak Company); levulose, for diabetics (C.P., Pfanstiehl); sucrose (standard sample no. 17, Bureau of Standards); maltose, purified malt sugar (Merck); xylose (Eastman Kodak Company).

Excised corn root tips of 0.2 cm. original length were grown individually in 125 cc. Erlenmeyer flasks containing 30 cc. of solution C plus 0.5 per cent agar and 2 per cent dextrose, 2 per cent levulose, or 1.66 per cent cane sugar. The dextrose and levulose were added to the medium before sterilization; the sucrose was separately sterilized in redistilled water and added to the agar and mineral salts which had also been separately sterilized. The cultures were incubated 84 days in the dark at room temperature. The results given in table I show dextrose to be somewhat superior to levulose or sucrose.

Root tips of approximately 2 cm. original length were grown in the modified Pfeffer's solution containing 2 per cent dextrose or 2 per cent cane sugar. Here also somewhat better growth was found in the solution containing dextrose.

Root tips of 1 cm. original length were grown individually in

125 cc. Erlenmeyer flasks containing 50 cc. of the modified Pfeffer's solution made up with 1 per cent dextrose, 1 per cent xylose, or a mixture of 1 per cent xylose and 1 per cent dextrose. The cultures were incubated in the dark for 81 days. The xylose was used by the roots to some extent but was less satisfactory than the dextrose and at this concentration appeared somewhat injurious. The roots in the dextrose solution grew more than twice the length of those in the xylose solution. The growth in the mixture of xylose and dextrose was superior to that in the xylose alone but much inferior to that in the dextrose alone.

TABLE I

GROWTH OF EXCISED CORN ROOT TIPS IN SOLUTION C PLUS 0.5 PER CENT AGAR WITH DEXTROSE, LEVULOSE, OR SUCROSE AS SOURCE OF CARBON. ORIGINAL LENGTH 2 MM. GROWN 84 DAYS IN DARK AT ROOM TEMPERATURE

CARBOHYDRATE	NO. ROOTS	AVERAGE FINAL LENGTH (CM.)	AVERAGE NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Dextrose.....	18	16.7	61	31.5	117	4.5	12
Levulose.....	17	11.9	33	23.5	77	5.5	1
Sucrose.....	15	12.2	21	26.0	72	3.3	1

The effectiveness of maltose as compared with dextrose was determined by growing root tips of 1 mm. original length in 30 cc. of a medium composed of solution CF plus 0.5 per cent agar and 2 per cent dextrose or maltose. Two root tips were grown in each flask and the cultures were incubated for 52 days in the dark at room temperature. The results summarized in table II show the inadequacy of maltose as a source of carbon for excised corn roots. The average growth in the maltose series was about one-fourth of that in the dextrose series. The roots in the maltose series became very thin, and if they attained a length of more than 3 cm. the tips had the diameter of a thin thread and broke easily when moved.

KNUDSON (6) grew entire plants in the light in the presence of various carbohydrates and found maltose less satisfactory than dextrose. The differences he found, however, were not so marked as those reported here, probably because the plants were supported in part by carbohydrates from the seed or grain and from photo-

synthesis. An excised root tip is limited almost completely to the carbohydrate supplied by the medium.

It is surprising that maltose should prove so much less satisfactory than dextrose. Maltose yields dextrose on hydrolysis and a considerable part of the food in the corn grain is starch which is hydrolyzed to maltose by amylase. It may be that the corn root does not produce maltase. It is possible also that the differences in growth result in part from contaminants in the sugars used. Sugars are difficult to secure free from mineral elements and nitrogenous substances, and these samples without doubt contained some foreign material.

TABLE II

GROWTH OF EXCISED CORN ROOT TIPS IN SOLUTION CF PLUS 0.5 PER CENT AGAR AND 2 PER CENT DEXTROSE OR MALTOSE. ORIGINAL LENGTH 1 MM. GROWN 52 DAYS IN DARK AT ROOM TEMPERATURE

CARBOHYDRATE	NO. ROOTS	AVERAGE FINAL LENGTH (CM.)	AVERAGE NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Dextrose.....	20	10.3	31	26.1	77	3.1	4
Maltose.....	16	2.8	1	6.0	5	0.5	0

In any event the experiments confirm the earlier conclusions that dextrose is a better carbohydrate for the growth of excised corn root tips than other sugars which might reasonably be considered and which can readily be secured.

Considerable differences have been observed in the effects of different brands of dextrose, however, and even different lots of the same brand, on the growth of excised corn root tips in solution C. This is illustrated by the following experiment in which seven different brands of dextrose were compared in solution C.

In this case root tips of 1 cm. original length were grown individually in 125 cc. Erlenmeyer flasks containing 30 cc. of solution C. The roots were grown 49 days at room temperature in the dark. Seven different samples of dextrose were used. Three times as much growth was secured in the culture solutions prepared with sample no. 1 as with sample no. 7 (table III).

We are not prepared to state the reason for the differences in the growth secured with the different brands of dextrose. Is it something organic or inorganic, something beneficial or detrimental? For example, is the difference between the effects of sample no. 1 (which gave the best results) and sample no. 7 (which gave the poorest) due to something beneficial in no. 1 or something detrimental in no. 7. These samples are both of high purity. The hydrogen-ion concentration of solution C prepared with these two samples was the same. Neither contained appreciable amounts of iron or of

TABLE III

GROWTH OF EXCISED CORN ROOT TIPS IN SOLUTION C PREPARED WITH DIFFERENT BRANDS OF DEXTROSE. ORIGINAL LENGTH 1 CM. GROWN 49 DAYS IN DARK AT ROOM TEMPERATURE

Brand of dextrose	Grade	No. of roots	Av. final length (cm.)	Av. no. secondary roots	Maximum length (cm.)	Maximum no. secondary roots	Minimum length (cm.)	Minimum no. secondary roots
1	C. P., special for injection.....	15	24.5	111	64.4	300	6.5	18
2	Granular.....	14	11.5	33	25.8	109	5.8	4
3	C.P.....	15	10.8	57	22.2	178	5.6	20
4	U.S.P.X.....	15	10.5	60	21.5	140	5.6	23
5	Analytical reagent	14	12.7	82	17.9	159	8.1	29
6	C.P.....	14	19.5	68	69.5	326	8.0	21
7	Special for injection.....	13	8.8	43	16.2	115	5.7	19

copper. The ash content of no. 1 was 0.008 per cent, somewhat greater than that of no. 7 which contained 0.003 per cent ash. This might suggest the presence of beneficial ash elements in no. 1 as the responsible factor, particularly as the ash content of sample no. 6 which gave good results was 0.034 per cent and that of no. 3 which gave poor results was 0.003 per cent. On the other hand, the ash of a 2 per cent solution of no. 1 is but 1.6 p.p.m. and the amount of ash added in the dextrose to 30 cc. of medium (the quantity customarily used for culturing a single root tip in these experiments) would be 0.048 mg. Any single element would make up but a fraction of this amount. It is not beyond the bounds of possibility, however, that the differences in the effects of the different brands of

highly purified dextrose are associated with the presence or absence of traces of the rarer elements. In any event the results are significant in emphasizing the importance of the character of the dextrose used in culture media. While minute attention may be devoted to the water or the mineral salts employed in culturing microorganisms, fungi, and other living things, investigators may pay little attention to the dextrose used, provided it is of high grade. It would be desirable to perform further experiments in which dextrose completely free of nitrogen and ash elements is used.

There remains the question as to whether sterilization of the solutions produced substances from the carbohydrate which were injurious to the roots. It is well known that dextrose is decomposed by heating in alkaline solutions, with the development of furfural, organic acids, and other products. SMITH (14) found that phosphates accelerated the decomposition. CONDREA and ROTH (2) and CIANCI and PANNAIN (1) found some decomposition occurred and some acid developed in the sterilization of dextrose in distilled water. The writers have confirmed this observation and also found that acid was formed from dextrose sterilized intermittently at 100° C. in a nutrient solution initially acid to neutrality.

It is not believed, however, that the limited growth and development of abnormalities in solution C are the result of decomposition products formed from the dextrose in sterilization. Comparisons of the growth of excised root tips in solution C prepared by sterilizing the dextrose and mineral salts separately and together at 100° C. on three successive days showed no significant differences. Further, the growth of roots was similar in solution C containing dextrose sterilized by heating at 100° C. and by filtration through a porcelain candle. This observation would seem to eliminate products formed from dextrose in sterilization as a factor of importance in determining the growth of excised roots in our experiments.

3. MINERAL SALTS.—The mineral salts used in solution C are significant from the standpoint of their purity, concentration, proportions, and adequacy.

The salts used in preparing the mineral nutrient solutions were of the customary chemically pure grade. In preparing solution C the following were used: $Ca(NO_3)_2$ (Merck White Label), $MgSO_4.7\ H_2O$

(Merck Reagent), KH_2PO_4 (Merck Reagent), $FeCl_3$ (Merck Reagent).

It is not thought that any contaminant in these chemicals is responsible for the poor growth of root tips in solution C. The growth in solution C prepared with the stock chemicals was compared with that in a similar solution made from salts purified by recrystallization.[3] Little difference was found in the effects of the two types of solutions.

The concentration of salts in solution C is low, totaling 70 p.p.m. It is not likely, however, that this is responsible for the results secured. No difference was found with the modified Pfeffer's solution which contains 642 p.p.m. of salts nor with solution C at five times (350 p.p.m.) its normal concentration. The concentration of dextrose remained constant at 2 per cent.

No experiments were performed in which the proportion of the various salts included in solution C was systematically varied, although it is recognized that proper balance is an important requirement in any nutrient solution. However, various nutrient solutions have been used (see section following on other nutrient solutions) and at one time or another the proportions of salts have been varied, particularly the ratio of calcium to magnesium, without material effect on the resulting growth. It is doubtful whether the salt proportions in solution C are of first importance, although such a statement cannot be made categorically without more extensive and systematic experiments.

The adequacy of the mineral salts in solution C should be considered particularly from the standpoint of the supply of iron and of various micro-essential elements.

In solution C iron is furnished as ferric chloride at a concentration of 1 p.p.m. Whether iron in this form and at this concentration is adequate may well be asked. WHITE (17) reports that ferric sulphate at 2.5 p.p.m. is more satisfactory than ferric chloride or ferric citrate.

While not prepared to answer the question finally at this writing, we do not think that the iron supply is the limiting factor in these experiments. We have substituted ferric tartrate and ferric citrate

[3] The purified chemicals were kindly prepared by Dr. ALBERT E. SAEGER.

for ferric chloride without marked effect, and have found additions to the medium (filter paper and water extract of filter paper) which add less than 0.2 p.p.m. of iron to improve growth decidedly.

An excised corn root tip is more limited in its stored mineral elements than is a root attached to a corn grain from which new supplies of ash elements may be translocated into the tip. It might be anticipated that a deficiency of one or more of the essential elements would be evidenced in the growth of excised root tips more readily than in the growth of roots attached to the grain. Although it was recognized that the chemicals used in preparing solution C contain traces of other elements as contaminants, there is still the possibility that the limited growth in that solution and similar solutions is the result of a deficiency in one or more of the rarer mineral elements.

Experiments were performed in which salts of manganese, boron, zinc, copper, and thallium were added to solution C. The growth of roots 1 cm. long in solution CF containing 0.1 p.p.m. each of manganese chloride, zinc chloride, and sodium borate was in some cases definitely better than in solution C. The improvement was not so great, however, as that secured by the addition of agar or of qualitative filter paper (see later paragraph). Root tips 1 mm. in length grew longer and produced more secondary roots in solution CF containing 0.5 per cent agar than in solution C containing agar.

The addition to solution CF of 0.001 p.p.m. or 0.01 p.p.m. of copper sulphate caused no improvement. Since a small decrease in growth was found with the addition of 0.01 p.p.m., it is not believed that further increases in the concentration of copper would prove beneficial. The addition to solution C of thallium nitrate at a concentration of 0.005 p.p.m. was without effect, although RICHARDS (7) has reported thallium beneficial to the growth of yeast.

While these experiments show some benefit from the addition of salts of manganese, boron, and zinc, they do not suggest that their addition to solution C would make it entirely satisfactory for the growth of excised corn roots. It is possible of course that the proper concentrations were not used. More extensive experiments with the rarer elements would be advisable, employing such mixtures as have been used by HOAGLAND and CHANDLER (4) and by TRELEASE and TRELEASE (15).

4. HYDROGEN-ION CONCENTRATION.—The hydrogen-ion concentration of solution C and of the modified Pfeffer's solution is near pH 4.5. The additions to Pfeffer's solution and to solution C which were found to be beneficial, such as agar, filter paper, yeast, peptone, or soluble starch (see later), make these solutions somewhat less acid although rarely changing the pH to a value higher than 5.4. There is therefore the possibility that the poor growth in solution C and in the modified Pfeffer's solution is due to too great an acidity. However, results of experiments in which excised corn root tips were grown in these solutions made less acid do not support such a hypothesis.

The modified Pfeffer's solution was adjusted to pH 5.0, 5.6, 5.9, and 6.1 by the addition of KOH, and excised root tips were grown for ten days in the solution. At the end of that time the tips were severed and transferred to fresh solutions of the same reactions. After two weeks the process was repeated. Little difference was noted in the growth of the root tips in the solutions of original pH 4.4, 5.0, and 5.6, although some reduction of growth was found at pH 5.9 and 6.1.

A repetition of this experiment in which potassium nitrate was omitted from the Pfeffer's solution and the concentration of salts was reduced showed no benefit in the less acid solutions. In this case the original reactions of the solutions were pH 4.4, 5.5, 6.8, and 7.1. The root tips in the two less acid solutions grew less well than those in the two more acid solutions.

The reaction of solution C was adjusted by the addition of dibasic potassium phosphate to acidities ranging from pH 4.5 to 5.2. Excised corn root tips 1 cm. long were grown individually in 30 cc. quantities of the medium in 125 cc. Erlenmeyer flasks at room temperature in the dark. In one experiment the salts and dextrose were sterilized together; in a repetition of it the salts and the dextrose were sterilized separately and mixed after sterilization. No significant differences were found in the growth at the different reactions, and none between the cultures in which the dextrose and salts were sterilized separately and those in which they were sterilized together.

That the hydrogen-ion concentration is not the cause of the limited growth in the modified Pfeffer's solution or in solution C was concluded from these experiments.

Aeration

In a liquid medium the oxygen available to the excised roots is that which dissolves in the medium, at least as soon as the roots sink below the surface of the liquid. Furthermore, the heating of the liquid in sterilization drives off all dissolved gases and, since the root tips were customarily placed in the culture flasks within a day or two after the sterilization of the medium, it is possible that the gases dissolved in the liquid were not in equilibrium with those of the air. The character of the commonest abnormality observed in the growth of the root tips suggests some interference with respiration, since the water-soaked condition is the result of the displacement by liquid of the gases normally present in the root.

Earlier experiments (11) in which excised root tips were grown in the light in the modified Pfeffer's solution through which a continuous current of air was bubbled did not suggest that the limited growth was due to a deficiency of oxygen. Additional experiments have been performed which bear upon this question. The results show that an increase in the oxygen supply over that secured in a liquid medium improves growth. They did not, however, demonstrate that the eventual stoppage of growth and the development of abnormalities can be entirely prevented by such means as were used to increase the aeration.

In the numerous experiments performed on the growth of excised root tips in liquid cultures, efforts were made to increase the oxygen supply to the root tip by tilting the culture flask in such a way as to drain the liquid from the root tip and expose it to the moist atmosphere in the flask. In other cases the root tip was pulled out of the liquid by a sterile transfer needle and left exposed to the air against the side of the flask. Tips were treated in these ways when they were observed to have become water-soaked and translucent and to have ceased or nearly ceased growth. In both methods the root tip was left exposed to the air until it was no longer translucent or until it had partially dried, before it was again immersed in the

solution. These treatments were sometimes but not always effective in causing the root to lose its water-soaked appearance and to renew its growth.

In order to investigate this systematically, an experiment was performed in which the flasks were tilted and the medium drained from the root tips at regular intervals. Roots were cut 2 mm. long and left for 24 hours on plates containing solution C plus 0.6 per cent agar. They were then grown individually in 30 cc. of solution C or solution C plus 400 p.p.m. of autolyzed yeast in 500 cc. Erlenmeyer flasks for 100 days at room temperature in the dark. For the first

TABLE IV

GROWTH OF EXCISED ROOT TIPS IN SOLUTION C OR IN SOLUTION C PLUS 400 P.P.M. AUTOLYZED YEAST IN 500 CC. ERLENMEYER FLASKS. ONE SET OF FLASKS TILTED TO EXPOSE ROOTS TO AIR AT REGULAR INTERVALS. ORIGINAL LENGTH 2 MM.

MEDIUM	TREATMENT	NO. ROOTS	AV. FINAL LENGTH (CM.)	AV. NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Solution C.....	None	15	5.4	30	8.5	36	3.7	7
	Tilted	12	11.5	47	45.6	194	5.4	8
Solution C. plus 400 p.p.m. autolyzed yeast .	None	12	7.6	30	19.2	70	4.2	5
	Tilted	13	12.0	44	24.6	87	5.0	20

30 days of the experiment (by the end of this time most of the roots had ceased growth) some of the flasks were tilted and the liquid drained from the root tip for approximately eight out of every 48 hours. Some improvement in growth was obtained by this treatment (table IV). The development of abnormalities in both solution C and solution C plus yeast was delayed and reduced by the tilting, although some water-soaked and swollen roots developed in the tilted series.

Some benefit, therefore, was derived from thus regularly exposing the roots to the air, but of course such treatment also removed them for the time being from the medium. To whatever the improvement of the roots was due, the treatment did not permit more than limited growth and did not eliminate the abnormalities.

It was thought that the depth of the liquid in the flasks might be of some importance. No significant difference was found, however, between the growth of roots in 30 cc. of liquid in 125 cc. flasks or the same quantity in 500 cc. flasks. No difference was observed between roots grown in 15 cc. and in 30 cc. of medium in the 125 cc. Erlenmeyer flasks. Such differences in oxygen supply as result from varying the depth of the liquid within the limits given above did not influence the growth of the root tips in solution C.

This should not be interpreted as indicating that oxygen supply is not a significant factor in the growth of excised corn root tips. A comparison of roots grown on the surface of an agar medium and

TABLE V

GROWTH OF EXCISED ROOT TIPS IN 30 CC. OF SOLUTION C PLUS 0.5 PER CENT AGAR. ORIGINAL LENGTH 1 CM. ROOT TIPS LEFT ON SURFACE OF MEDIUM IN ONE CASE; PRESSED TO BOTTOM OF MEDIUM IN OTHER CASE

POSITION	NO. ROOTS	AV. FINAL LENGTH (CM.)	AV. NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Surface.....	10	36.9	184	90.0	295	11.8	77
Below surface....	9	11.8	32	24.7	64	5.0	15

roots which had been pushed to the bottom of the flasks showed a decided difference in favor of the former.

Roots cut 1.0 cm. long were grown individually for 87 days in 30 cc. of solution C containing 0.5 per cent agar in 125 cc. Erlenmeyer flasks at room temperature in the dark. Some of the roots were left as usual on the surface of the agar, others were pressed gently through the medium to the bottom of the flasks. The results summarized in table V show that those roots pressed to the bottom of the flasks grew much less than those originally left on the surface of the agar. The difference in the roots grown on and under the agar is shown also in figure 4. Furthermore, three of the roots which had been pushed to the bottom of the flasks grew so that at some time during the experiment a portion of the root was exposed at the surface of the agar. These roots attained the greatest length and produced the largest number of secondary roots in the series under agar.

Besides the difference in final length and number of secondary roots, there was also a difference in the time at which secondary roots were formed in the two series. By the fourth day secondary roots had been formed by all those placed on the surface of the agar. On the roots which had been pressed to the bottom of the flasks branches developed on one root on the seventh day, on one on the tenth, on one on the thirteenth, on six on the nineteenth, and on one on the twenty-second day.

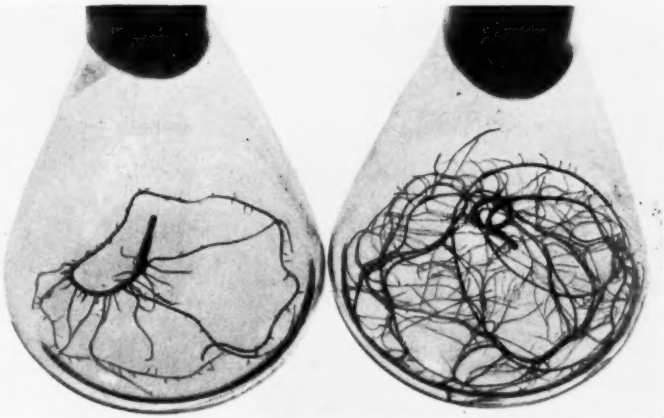


FIG. 4.—Typical roots grown 80 days in solution C plus 0.5% agar; original length 1 cm. Left, root which had been pushed through medium to bottom of flask; right, root left on surface of agar.

The marked difference in the growth of the two sets of roots is somewhat surprising because those originally on the surface soon grew down under the agar and the major portion developed beneath the surface.

Although the oxygen available to those roots grown under the agar was certainly no greater than that available in solution C without agar, the development of abnormalities was much less pronounced. For the first nineteen days no signs of abnormalities aside from a decrease in diameter and a deficiency of branches were observed in the roots grown under the agar. This is in contrast to the pronounced abnormalities which developed in solution C within the first few days of growth. On the nineteenth day a slight swelling

of the cortex at the basal end of some roots was observed. This was followed by a wrinkling of the cortex and the development of a translucent area near the basal end; but the roots under the agar did not develop the water-soaked appearance in the region of elongation of the main root, a condition so characteristic of many roots grown in solution C. The secondary roots were somewhat irregular in length and characteristically somewhat swollen, translucent, and stiff; the entire root systems of the members of the series under agar were slightly discolored. These abnormalities are to some extent the same as those observed in roots grown in solution C, but in the latter they appear earlier and are accentuated.

TABLE VI

GROWTH OF EXCISED CORN ROOTS IN SOLUTION C AND IN SAME SOLUTION PLUS 0.5 PER CENT AGAR. ORIGINAL LENGTH 1 CM. GROWN IN 30 CC. OF MEDIUM IN 125 CC. ERLENMEYER FLASKS FOR 57 DAYS IN DARK AT ROOM TEMPERATURE

MEDIUM	NO. ROOTS	AV. FINAL LENGTH (CM.)	AV. NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Solution C.....	17	6.9	22	9.7	41	5.5	3
Solution C+ 0.5% agar.....	20	34.2	178	92.0	448	4.0	20

AGAR.—In experiments performed earlier in this laboratory, superior growth of excised corn roots was secured in an agar medium. No attempt at that time was made, however, to compare the growth in a liquid medium with that on agar nor to determine the reasons for the beneficial effects of the agar. The writers have found that the growth of excised corn root tips is markedly improved and the percentage of abnormalities reduced by the addition of Difco agar to solution C.

The superiority of solution C containing sufficient agar to make a semi-solid medium is shown in table VI, in which excised root tips 1 cm. long were grown individually in 30 cc. of medium in 125 cc. Erlenmeyer flasks for 57 days at room temperature in the dark.

The beneficial effects of the agar are due to its water-soluble constituents and not to its colloidal character nor to the semi-solid

condition of the medium. This conclusion follows because a water extract of the agar added to solution C produces the same beneficial effect as the agar itself (fig. 5). The water extract was prepared by extracting 30 gm. of Difco agar twice with 500 cc. of redistilled water for 48 hours in the icebox and adding to solution C an amount of the filtered extract equivalent to 0.5 per cent agar.

No attempt was made to determine whether the beneficial effect of the agar was due to its organic or to its inorganic elements. The water extract of the agar made solution C somewhat less acid, raising the pH from 4.5 to 4.8. From other experiments in which the effect of hydrogen-ion concentration on the growth of excised roots

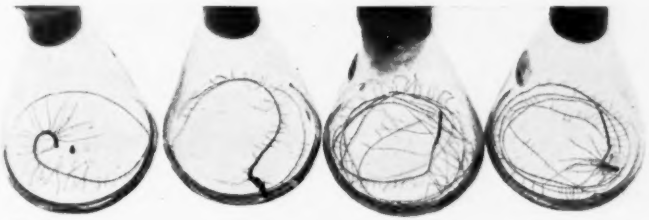


FIG. 5.—Effect of water extract of agar. From left to right: two best roots grown in solution C; two best roots grown in solution C plus water extract of agar. Original length 1 cm.; grown 47 days at room temperature in the dark.

in the modified Pfeffer's solution and in solution C was studied, it is not believed that the effect of the agar or of the water extract of the agar was due to its effect on the reaction of solution C.

FILTER PAPER.—Not only was a favorable effect obtained by the addition to solution C of agar or a water extract of agar, but the addition of a qualitative filter paper (Arthur H. Thomas 90 mm. circles no. 5160) proved even more beneficial. The beneficial effects of the filter paper were observed in solution C prepared with purified chemicals, solution C prepared with stock chemicals, solution CF, and the modified Pfeffer's solution. The presence of the filter paper markedly increased the growth in length and production of secondary roots, delayed and reduced the development of abnormalities, and improved the appearance and condition of the roots. While the optimum amount of filter paper was not exactly determined, one-

half or one full sheet of filter paper in 30 cc. of medium appeared to produce the best results. The following experiment (table VII) illustrates the effects of the filter paper. In this case excised corn root tips 1 cm. long were grown individually in 30 cc. of medium in 125 cc. Erlenmeyer flasks for 57 days in the dark at room temperature. The basic medium was solution C containing 2 per cent dextrose. The filter paper was added to the medium before sterilization in the proportion of one-eighth sheet, one-half sheet, or two sheets per flask. While a distinct benefit is evident (fig. 6) from the addition of one-eighth sheet, a much greater effect was secured from the addition of one-half sheet. Two sheets appear to be somewhat above the optimum.

TABLE VII

EFFECT OF ADDITION OF QUALITATIVE FILTER PAPER TO SOLUTION C ON GROWTH OF EXCISED CORN ROOT TIPS. ORIGINAL LENGTH 1 CM. GROWN 57 DAYS IN 30 CC. OF MEDIUM IN 125 CC. ERLENMEYER FLASKS IN DARK AT ROOM TEMPERATURE. SHEETS OF FILTER PAPER 9 CM. IN DIAMETER USED

AMOUNT OF FILTER PAPER	NO. ROOTS	AV. FINAL LENGTH (CM.)	AV. NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
None	12	8.8	61	12.5	87	5.1	23
$\frac{1}{8}$ sheet	14	26.7	165	68.2	333	7.4	24
$\frac{1}{2}$ sheet	12	52.6	223	88.3	320	12.1	64
2 sheets	12	44.0	172	88.1	376	14.7	70

The filter paper made solution C somewhat less acid, as may be noted in table VIII, in which the pH after sterilization and the average pH after the growth of the roots are given. A single sheet of the filter paper weighed about 480 mg. and contained approximately 1 mg. of ash.

The effect of the filter paper is due to its water-soluble constituents. This follows because a water extract of the filter paper added to solution C or to the modified Pfeffer's solution improved the growth of the roots about as much as did the filter paper itself.

It is probable that the beneficial effects of the filter paper are due to its ash constituents, since quantitative (ashless) filter paper

(Whatman no. 40, 110 mm. circles) added to solution C in the proportion of one-eighth sheet per flask had no effect and a water extract of quantitative filter paper was ineffective.

FIG. 6.—Effect of qualitative filter paper on growth of excised corn root tips. From bottom to top: two best roots in solution C; two best in solution C plus $\frac{1}{8}$ sheet filter paper; two best in solution C plus $\frac{1}{2}$ sheet; two best in solution C plus 2 sheets. Original length 1 cm.; grown 57 days in 30 cc. of medium at room temperature in the dark.

COTTON AND SOLUBLE STARCH.—The effects of the addition to solution C of cotton and soluble starch were also determined. Absorbent cotton in the proportion of approximately 170 mg. per flask

TABLE VIII

INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS OF SOLUTIONS USED IN EXPERIMENT SUMMARIZED IN TABLE VII

MEDIUM	INITIAL pH	AV. pH AFTER GROWTH OF ROOTS
Solution C	4.6	5.2
Solution C + $\frac{1}{8}$ sheet	4.75	5.4
Solution C + $\frac{1}{2}$ sheet	5.0	5.6
Solution C + 2 sheets	5.4	6.1

containing 30 cc. of solution C had little beneficial effect on corn root tips of 1 cm. original length grown at room temperature in the dark. The addition of 1 per cent Merck's soluble starch to solution

C improved the growth and reduced the percentage of abnormal roots. Neither the cotton nor the soluble starch materially changed the pH of solution C.

PRETREATMENTS.—The beneficial effects of the addition to solution C of agar, water extract of agar, qualitative filter paper, or water extracts of qualitative filter paper raised the question as to what a brief treatment with these materials might do. The corn grains were therefore germinated as usual on a water agar and the root tips severed. Some of the severed tips were transferred at once to the culture medium; others were placed for 24 or 48 hours on the

TABLE IX

EFFECT OF PRETREATMENT ON AGAR ON GROWTH OF EXCISED CORN ROOT TIPS. ORIGINAL LENGTH 1 CM. GROWN IN 30 CC. OF MEDIUM FOR 41 DAYS IN DARK AT ROOM TEMPERATURE

MEDIUM	PRETREATMENT	NO. ROOTS	AV. FINAL LENGTH (CM.)	AV. NO. SECONDARY ROOTS	MAXIMUM LENGTH (CM.)	MAXIMUM NO. SECONDARY ROOTS	MINIMUM LENGTH (CM.)	MINIMUM NO. SECONDARY ROOTS
Solution C.....	None	20	8.7	39	17.9	84	4.0	8
Solution C + 0.5% agar.....	None	11	30.8	148	68.3	340	7.8	21
Solution C.....	48 hours on agar	13	28.1	133	98.2	470	3.5	20

surface of a semi-solid agar medium or on moist filter paper. After such pretreatment they were transferred to the culture medium.

The pretreatment of 1 cm. root tips on agar for 24 or 48 hours was found to be decidedly beneficial although not so beneficial as the addition of agar or a water extract of agar to the medium. The excised root tips were pretreated in petri dishes on the surface of 0.5 or 0.75 per cent agar made up with solution C with or without dextrose. No experiments were performed in which the root tips were pretreated on a water agar. The results of a typical experiment are given in table IX. In this case excised root tips 1 cm. long were grown individually in 30 cc. of medium in 125 cc. Erlenmeyer flasks for 41 days in the dark at room temperature. The root tips were grown in solution C, in solution C plus 0.5 per cent agar, and in solu-

tion C after pretreatment on agar. The growth in solution C of the pretreated roots was almost as great as that of untreated roots grown in solution C plus 0.5 per cent agar and very much superior to the growth of untreated roots grown in solution C.

Pretreatment of root tips originally 1 cm. in length for 24 or 48 hours on the surface of qualitative filter paper moistened with solution C or solution C without dextrose in petri dishes was also found beneficial, although not so much so as the addition of filter paper to the medium. Pretreatment on filter paper seemed somewhat more effective than pretreatment on agar.

Although pretreatment of roots 1 cm. long was distinctly beneficial, that of roots 2 mm. long seemed to have little effect in the few experiments performed. Pretreatment was not always effective in preventing the development of abnormalities (fig. 3).

To what is the beneficial effect of pretreatments on agar or filter paper due? A number of possibilities suggested themselves and some of them were tested by experiment. The results indicate that the absorption by the roots of something from the agar or the filter paper is responsible for the benefits of the pretreatments.

It was thought that these beneficial effects might be due to the increased oxygen supply during the exposure on the agar or on the filter paper. If this is correct the same beneficial effects should be observed if the excised root tips were exposed in a moist atmosphere in the absence of agar or filter paper. However, such was not the case. The benefit derived from pretreatment on agar or filter paper did not appear to be a matter of increased supply of oxygen. Root tips 1 cm. long exposed for 16 hours in sterile petri dishes containing 2 cc. of redistilled water and transferred to solution C grew no better than those transferred to the solution at once after cutting. Root tips 1 cm. long were placed in sterile petri dishes containing a micro-slide and 4 cc. of solution C without dextrose. They were placed in the dishes in such fashion that the cut basal end rested on the slide in the moist air while the tip was in contact with the liquid. After 24 hours they were transferred to solution C. Here again the exposure to the moist atmosphere was not beneficial.

It was suggested that perhaps the agar or filter paper absorbed some toxic substances formed at the cut base of the excised root.

KEEBLE, NELSON, and SNOW (5) found that washing decapitated roots attached to the grain favored their growth. However, the writers washed excised roots of 1 cm. original length for 45 minutes using 10 cc. of sterile redistilled water for each root and found no benefit when the growth of the washed roots in solution CF was compared with that of roots transferred at once to the solution. Roots washed and then placed on filter paper for 24 hours showed the beneficial effect of the contact with filter paper when grown in solution C.

These experiments on pretreatment in a moist atmosphere or by washing with redistilled water did not support the idea that the beneficial effects of pretreatment on agar or filter paper were due to a greater oxygen supply or to the removal of toxic products from the cut surface. Nevertheless, a further series of experiments was performed in which the excised root tips which had been pretreated on filter paper were freshly cut before being placed in the culture solutions. If the cutting of the root tips in excising them was the cause of the poor growth because of shock or any other reason, and the pretreatment on filter paper in some way neutralized the bad effects, then it seemed that the beneficial effects of pretreatment on filter paper would be eliminated by the fresh cut. However, that was not the case. Excised root tips 1.0 cm. long were pretreated on filter paper for 24 hours. At the end of that time 3 mm. was cut from the base of some of them. All were transferred to solution C and little difference between the resulting growths of the two sets was found.

If the effect of pretreatments on agar or on filter paper is not the result of greater oxygen supply or of the neutralization of the effects of cutting the root, then it would seem that it must be due to the absorption by the root of some substance or substances from the filter paper or the agar. The question at once arises as to why 24 hours on agar after excision should permit more absorption than would occur during the two or three days' contact of root and agar during germination. It is necessary to assume that the materials absorbed from the agar by the root attached to the grain are translocated in part out of the root tip, while in the excised root they accumulate. This would explain why pretreatments are less bene-

ficial for excised root tips 0.2 cm. long than they are for root tips 1.0 cm. long. In the former case there is less opportunity for accumulation because of the smaller amount of tissue.

Although the writers are inclined to explain the beneficial effects of pretreatment on agar or filter paper as due to the absorption of substances from the agar or filter paper, one type of pretreatment found effective cannot easily be explained on that basis. This consisted of placing cut roots 1 cm. long vertically against the inside of a pyrex flask containing solution C in such fashion that the tip touched the liquid but the rest of the root was above the liquid. After 24 hours such roots were washed down into the solution and their growth compared with that of root tips which had been placed in the solution immediately after being severed from the grain. Although the number of roots treated by this method was small the benefits appeared unmistakable, the pretreated roots making between two and three times as much growth as the untreated roots.

OTHER NUTRIENT SOLUTIONS.—WHITE (**18**) has used a modification of Pfeffer's solution containing cane sugar and dried brewers' yeast for excised tomato root tips and has secured unlimited growth. He found Uspenski's solution also to be a favorable solution. These two solutions were compared by the writers with solution C to which autolyzed yeast had been added. In addition excised corn root tips were grown in the following solution adapted from one used by TRELEASE and TRELEASE (**15**):

KH_2PO_4	0.002M
$Ca(NO_3)_2$	0.002
$MgSO_4$	0.001
NH_4NO_3	0.001
Ferric citrate	0.00001

Sufficient K_2HPO_4 was added to this solution to bring the hydrogen-ion concentration to pH 5.2.

No difference in total length or production of secondary roots was noted between these solutions. In WHITE'S solution comparatively few roots became discolored but the main roots and many of the branches became very thin. In none of these solutions was the growth in length and number of branch roots comparable to that in

solution C which contained agar, agar washings, qualitative filter paper, or filter paper washings.

YEAST.—The beneficial effects of the addition of yeast (either autolyzed yeast or dried brewers' yeast) to the modified Pfeffer's solution originally reported by ROBBINS (9) was confirmed. Similar beneficial effects were noted also when yeast was added to solution C. From the experiments performed, yeast seemed somewhat more effective in solution CF than in solution C, and in solution CF plus agar than in solution C plus agar. The yeast was usually added at a concentration of 400 p.p.m. In one experiment, however, in which autolyzed yeast or dried brewers' yeast was added to solution CF plus 0.5 per cent agar, surprisingly beneficial effects were secured on the growth of 1 mm. root tips by the addition of as little as 1 p.p.m. of the yeast, the brewers' yeast being the more effective. Ten p.p.m. was somewhat less effective, and 50 p.p.m. although still beneficial was not so satisfactory as the 10 p.p.m. Twice as much growth was secured in solution CF plus 0.5 per cent agar containing 1 p.p.m. of dried brewers' yeast as in the medium without the yeast, and one root tip 1 mm. long grew in 71 days to a total length of 61.2 cm. and produced 175 secondary roots. These results are so unexpected that they should be confirmed.

Some experiments were also performed in which yeast ash was added to the modified Pfeffer's solution or to solution C. The yeast ash was dissolved in hydrochloric acid and evaporated to dryness or neutralized with sodium hydroxide. In all cases a benefit from the addition of the ash preparations was found. However, contaminants in the hydrochloric acid used or in the sodium hydroxide may have been responsible in part for the results.

GLUTATHIONE, PANTOTHENIC ACID, VITAMIN B.—Glutathione according to HAMMETT (3) stimulates cell division in roots attached to the seed or grain. The writers found no benefit on the growth of excised roots 1 cm. long from the addition of 0.192, 0.384, 0.96, 1.92, or 3.84 p.p.m. of glutathione to solution C plus 0.5 per cent agar. The roots were grown 55 days in the dark. Glutathione at a concentration of 1.92 p.p.m. did not improve the growth of root tips 2 mm. long in solution C plus 0.5 per cent agar.

WILLIAMS (16) has found pantothenic acid beneficial to the growth

of yeast. Pantothenic acid[4] (potency 70) was somewhat beneficial at a concentration of 50 p.p.m. to the growth of excised root tips 2 mm. long in solution C plus 0.5 per cent agar. In fact the longest root (85.0 cm.) from this original length grew on this medium. At a concentration of 16.6 p.p.m. no effect was noted. Some benefit was found on the growth of root tips originally 1 mm. long in solution CF plus 0.5 per cent agar from the addition of 10 p.p.m. of pantothenic acid (potency 133). No benefits were secured from 1 p.p.m. or from 50 p.p.m. The experiments with pantothenic acid were few in number; and although in some cases benefits were noted from the addition of this material, the results need confirmation.

SCHOPFER (**13**) has reported that vitamin B greatly improves the growth of Phycomyces. A sample of crystalline vitamin B was secured from WILLIAMS[5] and added to solution CF plus 0.6 per cent agar. No benefit was observed in the growth of root tips 1 mm. long from the addition of 0.1, 1, or 2 p.p.m. of vitamin B. The roots were grown for 50 days in the dark.

MAXIMUM GROWTH.—It will be of some interest to record the maximum growth of individual root tips of various original lengths found in the experiments which are discussed in this paper.

The maximum growth of excised corn root tips originally 1 mm. in length was obtained in solution CF plus 0.5 per cent Difco agar and 1 p.p.m. of dried brewers' yeast. In this medium at room temperature in the dark one root grew in 71 days to a total length of 61.2 cm. and produced 175 secondary roots. This is an increase of 0.9 cm. per 24 hours. In solution C plus 0.5 per cent agar the maximum growth was 26.1 cm. with 77 secondary roots.

For root tips 2 mm. long the maximum growth was secured in solution C plus 0.5 per cent agar and 50 p.p.m. pantothenic acid. In this medium at room temperature and in the dark one root grew in 61 days to a total length of 85.0 cm. and produced 244 secondary roots. This is an increase of 1.4 cm. per 24 hours. Long roots were found also in solution C plus 400 p.p.m. of autolyzed yeast (65.3 cm.) and in solution C plus 0.5 per cent agar (53.4 cm.). In solution

[4] Gratitude is expressed to Dr. R. J. WILLIAMS for supplying the material used.

[5] Gratitude is expressed to Dr. R. R. WILLIAMS for the gift of this material.

C after pretreatment on agar the maximum was 9.7 cm. with 45 secondary roots.

The maximum growth of root tips 10 mm. long was secured in solution C after pretreatment on agar. This root at room temperature in the dark grew in 41 days to a length of 98.2 cm. and produced 470 secondary roots, an increase of 2.4 cm. per 24 hours. Long roots were secured also in solution C plus 0.5 per cent agar (92.0 cm. with 448 secondary roots); solution C plus one-eighth, one-half, or two sheets of qualitative filter paper (85.5, 88.3, and 88.1 cm. respectively); solution CF plus 400 p.p.m. of autolyzed yeast (72.2 cm.). The maximum in solution C prepared with the Coleman and Bell dextrose was 25.2 cm. with 168 secondary roots, although a root 64.4 cm. long with 300 secondary roots was grown in solution C prepared with another brand of dextrose of high purity (Pfanstiehl C.P. for injection).

Discussion

Why do excised corn root tips develop abnormalities and show limited growth in the mineral solutions containing dextrose which were used in these experiments? Conversely, how can excised corn root tips be maintained in a normal and growing condition indefinitely? There is no *a priori* reason why this is not possible of accomplishment. These questions cannot be answered categorically as a result of the experiments reported in this paper, but a discussion of the possibilities is important from the standpoint of further investigation of this problem.

The possible causes for the development of abnormalities and the limited growth in solution C or similar solutions might be enumerated as follows:

1. An injurious condition in the medium because of the presence of something which is toxic, because of an unbalanced condition or some other condition.

2. A nutrient deficiency in the medium which might be lack of sufficient oxygen, a mineral nutrient, or some essential organic substance or substances which the root does not synthesize.

3. The condition of the excised root at the time of transfer; a condition resulting from its genetic constitution, its previous history, or

from some phase of the treatment in preparing or excising the root tip.

4. Some environmental factor not included in the medium (for example, temperature of incubation).

If the results are due to an injurious condition in the medium, the distilled water would not seem to be at fault since little difference was found in the effects of different kinds of distilled or redistilled water. It would not appear to be due to the hydrogen-ion concentration, which was normally near pH 4.5, since making the solutions used less acid by the addition of KOH or K_2HPO_4 did not improve conditions. It would not appear to be due to any contaminant in the mineral salts used since purified salts gave no better results than the stock chemicals. It is not due to changes in the dextrose caused by heating in sterilization since dextrose filtered sterile was no better than dextrose sterilized by heat. It is possible that some contaminant in the dextrose is responsible. This is not probable since samples of dextrose of high purity were used and further, cane sugar of highest purity was not superior to dextrose when substituted for the latter. It may be due to a lack of balance in the mineral solution although some variation in the proportions of salts was without marked benefit. In fact we are not inclined at this time to ascribe our results to any toxic condition in solution C, in the modified Pfeffer's solution or in similar solutions, although it is recognized that limited growth and abnormalities can be produced by toxic substances.

It does not seem likely that the limited growth and abnormalities result from the treatment of grains or roots before placing the root tips in the culture flasks. This treatment includes the sterilization of the grains, their germination, the excision of root tips and their transfer to the culture flasks. That sterilization of the grains is not a factor is indicated by the results reported by ROBBINS and MANEVAL (10) with roots secured from unsterilized grains, and by the fact that roots of grains grown in water culture show no differences whether sterilized or not sterilized with calcium hypochlorite. The temperature of germination was 26° C., and the medium (water agar) upon which the grains germinated was favorable for growth, as demonstrated by the appearance of the roots and by the cultures

to which agar or agar washing had been added. The conditions of germination, therefore, do not appear to be involved. Neither does the cutting of the root seem important. No difference was found in the effects on roots of the lengths used in these experiments of a thin or a thick knife blade used in excising the roots. Washing the roots after excision or the removal of a fresh piece of the root after pretreatment did not affect the resulting growth.

The environmental conditions other than the culture medium itself do not seem to be involved. The temperature at which the cultures were incubated was not controlled and varied between 19° and 30° C. with an average of 25° C. The higher temperatures are unfavorable but most of the culture work was carried on during the fall, winter, and spring when the temperatures were from 20° to 22° C. Light under some circumstances is a favorable factor (**11**). It does not, however, overcome the effects of solution C or similar solutions.

The osmotic pressure of our solutions is about 0.11 mol., less than half the molecular concentration which causes plasmolysis of corn root hairs (**12**).

The possibility that the limited growth in solution C or similar solutions is the result of a deficiency in the medium seems most probable. If such is the case then it is not due to a deficiency of water since the roots are grown in liquid cultures, nor to a lack of carbohydrate since there is a large amount of dextrose in the solution at the termination of the experiments. A deficiency of oxygen is of some significance. This is shown by the beneficial effects of exposing root tips to the air by tilting the flask or by pulling the root tips temporarily out of the liquid medium. Lack of oxygen is not alone responsible for the results in solution C, however, since the addition to the liquid medium of agar washings, qualitative filter paper, qualitative filter paper washings, soluble starch, and the use of certain brands of dextrose produce more improvement than any of the methods used to increase the oxygen supply to the roots. It is possible, of course, that the additions referred to facilitate the respiratory activity of the root even though the oxygen supply is actually not increased. If the limited growth and development of abnormalities in solution C or similar solutions are due to a de-

ficiency in the medium it is a deficiency which is supplied wholly or in part by peptone, yeast, agar, filter paper, soluble starch, and some contaminant in certain brands of dextrose. It is difficult although not impossible to conceive of these various materials supplying an organic deficiency; a mineral deficiency seems more likely. If such is the case it is not a deficiency of phosphorus, sulphur, magnesium, potassium, calcium, copper, or thallium. It is probably not a deficiency of iron. Some improvement was observed by the addition of a mixture of salts of manganese, boron, and zinc but not as much as by the addition of agar or filter paper. Further experiments should be performed in which it is definitely determined whether the beneficial effects of peptone, yeast, agar, filter paper, and soluble starch are due to organic or to inorganic constituents; and the effect of various rarer elements should be tested.

It is entirely possible that more than one factor may be found important in making a synthetic solution such as solution C adequate for the growth of excised corn root tips. Not only may mineral elements other than those contained therein be necessary, but organic nitrogen (such as amino acids) or other organic substances may be significant.

Are any of the various media used in these experiments entirely adequate for the growth of excised corn roots? It is probable that one or more of them is, although the solution found by WHITE (**18**) as satisfactory for unlimited growth of tomato roots does not appear from our results satisfactory for excised corn root tips. However, in these experiments we have not attempted to investigate directly the problem of unlimited growth of excised corn roots. It may be pointed out in this connection that not only do the plants used by WHITE and ourselves differ but that we have been concerned with the meristem of the main root while WHITE has used meristems of secondary roots as well. The use of the growing points of secondary roots greatly augments the possibilities of the continued growth of tissue from a single isolated tip by multiplying many times the number of cultures containing meristem, and thus permitting the continuance of cultures from a root in which the meristem of the primary root tip has ceased to grow. In addition, the use of subcultures serves a selective process by eliminating at the start those

roots which for some genetic or other reason do not adapt themselves to growth in the medium used.

The possible advantage of the cultivation of excised root tips or other tissues of higher plants for the investigation of the nutritional requirements of higher plants and their development is obvious. An excised root tip in contrast to a seed is far more limited in its stored supply of mineral nutrients and organic constituents, and furthermore is not supplied during its development by the products formed by the photosynthetic activity of the leaves. By proper refinements of technique it seems entirely feasible to contribute by these methods to the problems involved in the mineral nutrition of higher plants, the synthesis of proteins, and the possible function of accessory growth substances.

Summary

1. Excised corn root tips grown in a liquid medium (solution C) composed of calcium nitrate, magnesium sulphate, potassium dihydrogen phosphate, ferric chloride, and dextrose showed limited growth and sometimes became abnormal. Similar results were secured in a modified Pfeffer's solution containing dextrose.

2. The amount of growth in solution C varied with the brand of dextrose used, even when the dextrose was of high purity.

3. The substitution of levulose, cane sugar, xylose, or maltose for dextrose in solution C was of no benefit; in fact, the growth with xylose and maltose was very poor.

4. Growth was improved by the addition to solution C of Difco agar, water extracts of agar, qualitative filter paper, water extracts of qualitative filter paper, soluble starch, autolyzed or dried brewers' yeast, or a mixture of salts of manganese, boron, and zinc.

5. The best results were secured by the add tion of qualitative filter paper in the proportion of one half sheet, 9 cm. in diameter, to 30 cc. of medium.

6. Exposure of excised root tips originally 1 cm. long for 24 or 48 hours on the surface of a semi-solid agar medium or on moist qualitative filter paper markedly favored their later growth in solution C.

7. Exposure of excised root tips in a moist atmosphere or washing them in redistilled water before cultivation in solution C was not beneficial.

8. Recutting excised roots after pretreatment on filter paper did not affect their later growth in solution C.

9. Increased aeration of the root tips in the liquid medium increased the growth and delayed the occurrence of abnormalities. The results were not so favorable as those secured by the addition of agar or of filter paper to the medium.

10. Reducing the acidity of the solution, increasing its salt concentration, substituting ferric tartrate or ferric citrate for ferric chloride, adding quantitative filter paper, absorbent cotton, copper sulphate, thallium nitrate, glutathione, or vitamin B to the medium were not beneficial.

11. The addition of pantothenic acid was in some cases beneficial.

12. Pfeffer's solution, Uspenski's solution, and the solution used by WHITE for the cultivation of excised tomato roots were no more effective than solution C.

13. The maximum growth of individual excised corn root tips 1 mm. long was 61.2 cm., or 0.9 cm. per 24 hours; of root tips 2 mm. long was 85.0 cm., or 1.4 cm. per 24 hours; of root tips 10 mm. long was 98.2 cm., or 2.4 cm. per 24 hours.

14. It is thought that the poor growth in solution C and similar solutions is the result of a deficiency in the solution, a part of which at least is inorganic.

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EFFECT OF ALTERNATE MOISTENING AND DRYING ON GERMINATION OF SEEDS OF WESTERN RANGE PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 475[1]

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(WITH NINE FIGURES)

Introduction

Although considerable study has been given to the germination behavior of various seeds, little is known regarding the effect that alternation of moistening and drying has upon their germination. In times of drought the seeds dry out and lie in that condition for an indefinite period. In preliminary tests conducted with the seeds of a large number of range plants from the Wasatch Plateau in central Utah, some of the seeds gave high percentages of germination under ordinary germinating conditions with continuous moisture at room temperature; others gave little or no germination under such conditions.

REVIEW OF LITERATURE

The literature includes few references concerning the seeds of range plants or the effect of drying after the seeds have absorbed water. SAMPSON (**29, 30, 31, 32**) reports the percentage of viability in germination tests of the seeds of a number of range plants[2] from

[1] In cooperation with the Intermountain Forest and Range Experiment Station of the United States Department of Agriculture, Forest Service.

[2] Owing to changes in nomenclature or in the check identification of certain species from the Manti National Forest by the Bureau of Plant Industry, ten of the species studied by SAMPSON are discussed in this report under other names:

Agropyron pauciflorum (Schwein.) Hitchc. (*A. tenerum* Vasey) not *A. violaceum* (Hornem.) Lange
Bromus anomalus Rupr. (*B. porteri* [Coult.] Nash.)
Bromus polyanthus Scribn. (*B. marginatus* Nees)
Melica bulbosa Geyer (*M. bella* Piper)
Poa secunda Presl. (*P. sandbergii* Vasey)
Stipa columbiana Macoun (*S. minor* Scribn.)

[Footnote continued on p. 244]

the Manti National Forest in central Utah, and the Wallowa National Forest in northeastern Oregon. Fourteen of the species which he studied (seven of which were from the Manti National Forest) are included in this investigation. In the tests conducted by SAMPSON most of the seeds gave low percentages of germination, but unless the conditions requisite for germination of each particular species are supplied, the results do not show the true viability of the seeds. The lack of adequate data regarding the conditions used in SAMPSON'S tests makes a comparison of results impossible.

Several investigators have studied the effect of reduced moisture content on germination, using fresh seeds for desiccation. EWART (**10**) concluded that the moisture content could not be reduced below 2 or 3 per cent without seriously affecting the vitality of the seeds. Others (**9, 14, 25, 28, 33, 35**) found that the water content could be reduced below this point, in some instances to a mere trace of moisture, with little or no effect on subsequent germination. ATTERBERG (**1**) observed that drying immature barley seeds at 37° C. increased the percentage of germination over seeds dried at lower temperatures, but drying soaked seeds of high germinative capacity at 48° to 50° C. or higher, reduced the percentage of germination.

From experiments with *Brassica alba*, KIDD (**20**) and KIDD and WEST (**21**) discovered that drying seeds during an early stage of development previous to germination accelerated germination when the seeds were remoistened, but drying the seeds after cell division had advanced resulted in injury. They found also that secondary dormancy was broken by complete redrying, or by removal of the seed coat, followed by remoistening. They interpret such germination as the result of mechanical stimulus on the embryo and not as changes within the seed coat, although removing the coats gave 100 per cent germination within a day after moisture was supplied. The effect of desiccation on the seed coats of certain water plants (**4, 23**) has long been known to be beneficial to germination.

The seed coat, according to CROCKER (**3, 4, 5, 6**), is most frequent-

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Artemisia incompta Nutt. not *A. discolor* Dougl.

Lepidium densiflorum Schrad. (*L. ramosissimum* A. Nels.)

Pentstemon rydbergii A. Nels. not *P. procerus* Dougl.

Pseudocymopterus montanus (A. Gray) Coult. and Rose (*P. tidestromii* Coult. and Rose).

ly the cause of delayed germination. It may prevent the absorption of water (**11, 13, 15, 16, 26**) or oxygen (**2, 3, 34**), or may mechanically restrain the embryo from developing (**6, 7**). The seeds of *Amaranthus retroflexus* (**6**), *Avena fatua* (**2, 36**), and some of the grains (**1, 22**) have been found to germinate better after a period of dry storage. CROCKER interprets the changes in *Amaranthus retroflexus* as due to hysteretic changes in the colloids of the seed coat, which lower the elasticity and the breaking strength of the coat (**6**). Changes in other seeds might harden the seed coat or affect the permeability to oxygen or water, as observed by DUVEL (**9**). Not only the seeds of different species, but the seeds of a given species may also vary in their oxygen requirement (**3, 34**). CROCKER (**3**) found that the rate of diffusion of oxygen through the seed coat of *Xanthium* was increased when the coat was allowed to become somewhat dry.

MUNERATI and ZAPPAROLI (**24**) investigated the effect of alternately moistening and drying the seeds of several noxious weeds. For 17 months they subjected the seeds to short (15 days) and long (3 months) periods of drying following a 15-day period of moistening. In four species germination was increased materially by both short and long periods of drying, while with two species there was only a slight increase. Drying decreased the germination of one species and had no effect upon another.

PURPOSE OF INVESTIGATION

The purpose of this investigation was to seek information concerning the following questions: What effect would an alternation of moistening and drying for short periods have upon the seeds of the different species of range plants? Would it hasten or delay the germination of seeds exposed to such conditions? Would it affect the percentage of germination? Might it have a beneficial effect upon the germination of seeds which gave little or no germination with continuous moisture at room temperature, or would the seeds be injured? Would seeds dried after being in moist conditions for some time without germinating, still retain their viability; that is, would seeds in which the processes of after-ripening and embryo development were interrupted by drying, germinate when again supplied with moisture?

Materials and methods

Species studied

Many of the "seeds" studied are fruits rather than seeds, but for the sake of convenience, the terms seed and seed coat are used regardless of the origin of the enveloping structures. Seeds of the following 42 species[3] were chosen to study the effect of alternate moistening and drying for short periods. For the long period of drying only 25 of the 42 species were used.

Grasses.—*Agropyron pauciflorum* (Schwein.) Hitchc. (*A. tenerum* Vasey), *Bromus anomalus* Rupr. (*B. porteri* [Coult.] Nash), *B. polyanthus* Scribn., *Hordeum nodosum* L., *Melica bulbosa* Geyer, *Poa interior* Rydb., *P. secunda* Presl. (*P. sandbergii* Vasey), *Stipa columbiana* Macoun, and *S. lettermani* Vasey.

Herbs other than grasses.—*Achillea lanulosa* Nutt., *Androsace diffusa* Small., *Artemisia incompta* Nutt., *Castilleja sulphurea* Rydb., *Chenopodium album* L., *Collomia linearis* Nutt., *Delphinium barbeyi* Huth., *Erythronium parviflorum* (S. Wats.) Goodding, *Frasera speciosa* Dougl., *Geranium viscosissimum* Fisch. & Mey., *Gilia pulchella* Dougl., *Lepidium densiflorum* Schrad., *Lupinus parviflorus* Nutt., *Moldavica parviflora* (Nutt.) Britton, *Orthocarpus tolmiei* Hook & Arn., *Pentstemon rydbergii* A. Nels., *P. subglaber* Rydb., *Plantago tweedyi* A. Gray, *Polemonium foliosissimum* A. Gray, *Polygonum douglasii* Greene, *Pseudocymopterus montanus* (A. Gray) Coult. & Rose, *Rudbeckia occidentalis* Nutt., *Rumex mexicanus* Meisn., *Sophia hartwegiana* (Fourn.) Greene, *Vicia americana* Muhl., and *Viola linguaefolia* Nutt.

Woody species.—*Acer grandidentatum* Nutt., *Cercocarpus montanus* Raf., *Chrysothamnus lanceolatus* Nutt., *Ribes inebrians* Lindl., *R. montigenum* McClatchie, *Sambucus microbotrys* Rydb., and *Symphoricarpos oreophilus* A. Gray.

The form of *Agropyron pauciflorum* studied is the wheatgrass heretofore incorrectly known as *A. violaceum*. *Bromus polyanthus* is considered by Hitchcock (17) to be only a form of *B. carinatus*, but

[3] Identification of all species has been checked with specimens in the herbarium of the Great Basin Branch Station, which have been identified by the Bureau of Plant Industry; or when there has been any question of doubt, the plants themselves have been checked by the Bureau of Plant Industry.

in accordance with the older usage, it is here treated as a species. *Achillea lanulosa* includes the subspecies *A. lanulosa alpicola* Rydb.

Collection and storage

The seeds were collected from plants in their natural habitat after they had ripened but prior to dissemination, except a few seeds of *Collomia*, *Polygonum*, and *Viola* which were taken from the ground soon after dissemination. The collections were made between August 16 and September 17, 1928, in the region of the Great Basin Branch of the Intermountain Forest and Range Experiment Station on the Manti National Forest in central Utah. The seeds of each species were collected from definite areas. In the case of *Poa secunda*, collections were made from two areas. These were kept separate and are designated as *P. secunda* no. 1 and *P. secunda* no. 2. The seeds from dry fruits were placed directly in paper containers. Those from fleshy fruits were washed free from pulp and air-dried before they were placed in paper containers. All seeds were later placed in bottles with cork stoppers and stored at room temperature.

Germination tests

The germination tests were made in the laboratories of the University of Chicago during a period extending from December 1928 to February 1930.

In preparation for these tests the seeds were disinfected in a 0.25 per cent solution of uspulun for 30 minutes and then rinsed in sterile water twice for 15 minutes each. They were then placed on sterile, moist cellucotton in petri dishes. Usually 50 seeds of a given species were used in each test, but for *Erythronium*, *Frasera*, *Geranium*, *Lupinus*, *Polygonum*, *Vicia*, *Acer*, and *Cercocarpus* 20 or 25 seeds were often used. As soon as the seeds germinated the seedlings were counted and discarded. Seeds were considered to have germinated when the hypocotyl protruded through the seed coat to an extent of 5 mm. or more.

To study the effect of short periods of alternate moistening and drying on the germination of the seeds included in this investigation, three sets of seeds were used, all of which were kept at room temperature. During the period of the experiment the temperature of the

room varied between 22° and 29° C. The temperatures used for drying are considerably lower than those used by ATTERBERG in his experiments with barley.

In one set of seeds (*A*), the substrate was kept moist continuously during the period of the test. In the second set (*B*) the substrate was kept moist for 5–7 days, then allowed to dry out by opening the petri dishes to permit evaporation. The dishes were left open until the cellucotton appeared thoroughly dry, then the substrate was remoistened and the dishes closed for three days, after which they were opened and the contents again allowed to dry. This process of alternate moistening and drying was repeated until the test had run for 51 to 53 days. Each period of drying required two or three days, depending upon the humidity of the atmosphere at the time. As an aid in preventing mold and bacterial growth, the petri dishes were kept in a case with cloth-covered sides which permitted the circulation of air but kept out dust and other foreign material.

The third set of seeds (*C*) was also subjected to alternate moistening and drying, but the period of moistening preceding the period of drying was shorter than that used in test *B*. Drying also took place more slowly. This set of seeds was not placed in conditions for germination until after the close of test *B*. The seeds were prepared as for *A* and *B* and placed on moist cellucotton for one day, after which the petri dishes were opened to dry. A period of high humidity prevailed at the time; consequently evaporation took place slowly and 12 days were required to dry out the substrate and seeds. They were then remoistened for one day and again allowed to dry out, which required four days. After a further period of three days under moist conditions the test was discontinued.

At the end of the period of alternate moistening and drying in both tests *A* and *B*, many of the seeds which had not germinated still looked healthy. With the exception of two species, *Lepidium* and *Rumex*, the seeds which had not germinated in test *B* were placed immediately in other conditions for germination to determine whether they had retained their viability. The seeds of *Lepidium* and *Rumex* were left in alternating moisture conditions for an additional 20 days. The seeds of the remaining species from test *B* were

remoistened, placed at 0° to 3° C. for eight days, then changed to room temperature of 22° to 29° C. for 12 days. At the end of the 12-day period the seeds of all but the following ten species were discarded. The seeds of *Delphinium*, *Pentstemon subglaber*, and *Ribes inebrians* were dried and kept for a later test. The seeds of seven species, *Collomia*, *Erythronium*, *Frasera*, *Gilia*, *Orthocarpus*, *Polemonium*, and *Polygonum*, were left for a further period of 120 days in a Minnesota germinator at 22° to 29° C. Following this long period at warm temperatures *Collomia* and *Frasera* were placed directly at alternating temperatures. The seeds of the other five species, *Erythronium*, *Gilia*, *Orthocarpus*, *Polemonium*, and *Polygonum*, were placed at cold temperatures of 0° to 3° C. for 34 days, then placed at alternating temperatures.

From test *C* the seeds of only five species were kept for further study. *Agropyron*, *Hordeum*, *Rumex*, *Viola*, and *Symphoricarpos* seeds were dried for 105 days to be used with other seeds in the following test.

To determine the effect of a long period of drying upon seeds which had been in moist conditions for some time, seeds which had failed to germinate under various conditions extending from 21 to 204 days were air-dried and kept at room temperature in paper containers for 105 days. Forty-eight samples representing 25 species were used. Three of the 48 samples were from the set of seeds subjected to alternating moisture conditions for 51 to 53 days (test *B*) and five samples were from those seeds which had been subjected to alternating moisture conditions for 21 days (test *C*), as previously noted. The remaining 40 samples were taken from seven other germination tests in all of which the seeds were kept moist continuously, but in which different temperature conditions had been used.

After drying for 105 days, the seeds were disinfected as for other tests and placed in petri dishes on sterile moist cellucotton. The seed coats of one set of *Vicia* seeds were broken by filing before they were placed in moist conditions. The seeds of each species were then placed under temperature conditions which were known or considered to be favorable for germination. The following were the temperature conditions used:

TABLE I

EFFECT OF ALTERNATE MOISTENING AND DRYING OF SEEDS ON GERMINATION

SPECIES	PERCENTAGE GERMINATION			
	WITH CONTINUOUS MOISTURE	UNDER ALTERNATING MOISTURE CONDITIONS		MAXIMUM GERMINATION OBTAINED IN OTHER TESTS
		RAPID DRYING	SLOW DRYING	
	TEST A	TEST B	TEST C	
Grasses				
Agropyron pauciflorum	6	2	4	86
Bromus anomalus	94	80	94	100
B. polyanthus	98	100	98	100
Hordeum nodosum	0	0	0	100
Melica bulbosa	0	0	0	94
Poa interior	96	98	80	100
P. secunda no. 1	64	68	40	86
P. secunda no. 2	16	20	2	58
Stipa columbiana	38	34	32	84
S. lettermani	10	26	16	98
Herbs other than grasses				
Achillea lanulosa	82	98	36	100
Androsace diffusa	52	62	10	38
Artemisia incompta	58	.....	76	86
Castilleja sulphurea	0	0	2	10
Chenopodium album	12	10	2	22
Collomia linearis	0	0	0	62
Delphinium barbeyi	0	0	0	2
Erythronium parviflorum	0	0	0	70
Frasera speciosa	0	0	0	45
Geranium viscosissimum	100	95	85	100
Gilia pulchella	0	0	0	32
Lepidium densiflorum	48	90	48	100
Lupinus parviflorus	95	70	75	100
Moldavica parviflora	2	2	0	4
Orthocarpus tolmiei	0	0	0	42
Pentstemon rydbergii	4	16	0	30
P. subglaber	0	2	0	22
Plantago tweedyi	80	84	98	100
Polemonium foliosissimum	0	0	0	30
Polygonum douglasii	0	0	0	5
Pseudocymopterus montanus	86	72	46	88
Rudbeckia occidentalis	60	44	90	98
Rumex mexicanus	22	52	0	100
Sophia hartwegiana	2	2	2	6
Vicia americana	0	5	5	100
Viola linguaefolia	0	0	0	2
Woody species				
Acer grandidentatum	0	0	0	53
Cercocarpus montanus	5	10	10	65

TABLE I—*Continued*

Species	Percentage germination			
	With continuous moisture	Under alternating moisture conditions		Maximum germination obtained in other tests
		Rapid drying	Slow drying	
	Test A	Test B	Test C	
Chrysothamnus lanceolatus	86	64	48	80
Ribes inebrians	0	0	0	98
R. montigenum	0	0	0	17
Sambucus microbotrys	0	0	0	6
Symphoricarpos oreophilus	0	0	0	32

1. Cold, 0° to 3° C. for the period of the test.

2. Alternating temperatures, 0° to 3° C. and 17° to 22° C. for approximately 12 hours each during the period of the test.

3. Cold, 0° to 3° C. for 8 days, then 22° to 27° C. until the end of the test.

4. Cold, as preceding for 8 days, then alternating temperatures as above until the end of the test.

The seeds were left in these conditions for 76 to 91 days.

Results

Tables I to IV give the results of the tests on the effect of short periods of alternate moistening and drying on the germination of the seeds included in this investigation. Table I gives for each species the various percentages of germination. The last column gives the maximum percentage of germination obtained in various other tests made from the same lots of seeds during a period extending from December 1928 to February 1932. The complete results of these tests will be published in a subsequent paper. While the results in the last column do not give a true index of viability for all of the species studied, they do provide a basis for comparison with the results obtained from the seeds under conditions of alternating moisture.

The seeds of the following 16 species gave no germination at room

temperatures, either with continuous moisture or with alternate moistening and drying. *Acer grandidentatum, Collomia linearis, Delphinium barbeyi, Erythronium parviflorum, Frasera speciosa, Gilia*

TABLE II

COMPARISON OF PERCENTAGE AND TIME OF GERMINATION OF SEEDS UNDER THREE CONDITIONS OF MOISTURE

Species	Germination					
	Continuous moisture		Alternating moisture conditions			
			Rapid drying		Slow drying	
	Days required	Percentage	Days required	Percentage	Days required	Percentage
Grasses						
Bromus anomalus	5–25	94	3–34	80	4–14	94
B. polyanthus	3–16	98	3–25	100	4–12	98
Poa interior	3–13	96	3– 8	98	4–21	80
P. secunda no. 1	3–37	64	3–16	68	4–10	40
P. secunda no. 2	5–30	16	5–16	20	14	2
Stipa columbiana	3–39	38	3–25	34	4–12	32
S. lettermani	22–37	10	11–34	26	7–18	16
Herbs other than grasses						
Achillea	2–52	82	4–28	98	7–21	36
Androsace	4–26	52	9–52	62	7	10
Artemisia	4–46	58	.....	.....	4–14	76
Chenopodium	2– 9	12	2–12	10	21	2
Geranium	3–18	100	5–20	95	4–21	85
Lepidium	3–51	48	3–53	90	4–21	48
Lupinus	5–53	95	10–48	70	4–21	75
Pentstemon rydbergii	9–23	4	13–48	16	.....	0
Plantago	3– 6	80	7–13	84	4–18	98
Pseudocymopterus	9–28	86	13–29	72	12–21	46
Rudbeckia	3– 9	60	7–10	44	4–13	90
Rumex	23–25	22	24–53	52	.....	0
Woody species						
Chrysothamnus	6–49	86	10–44	64	4–21	48

pulchella, Hordeum nodosum, Melica bulbosa, Orthocarpus tolmiei, Polemonium foliosissimum, Polygonum douglasii, Ribes inebrians, R. montigenum, Sambucus microbotrys, Symphoricarpos oreophilus, Viola linguaefolia.

The following seven species gave from 2 to 10 per cent only: *Agropyron pauciflorum, Castilleja sulphurea, Cercocarpus montanus,*

Moldavica parviflora, *Pentstemon subglaber*, *Sophia hartwegiana*, *Vicia americana*.

The time required for the germination of the seeds in tests *A*, *B*, and *C*, with the percentage of germination obtained for each of the 19 species which gave over 10 per cent germination at room temperatures, is shown in table II.

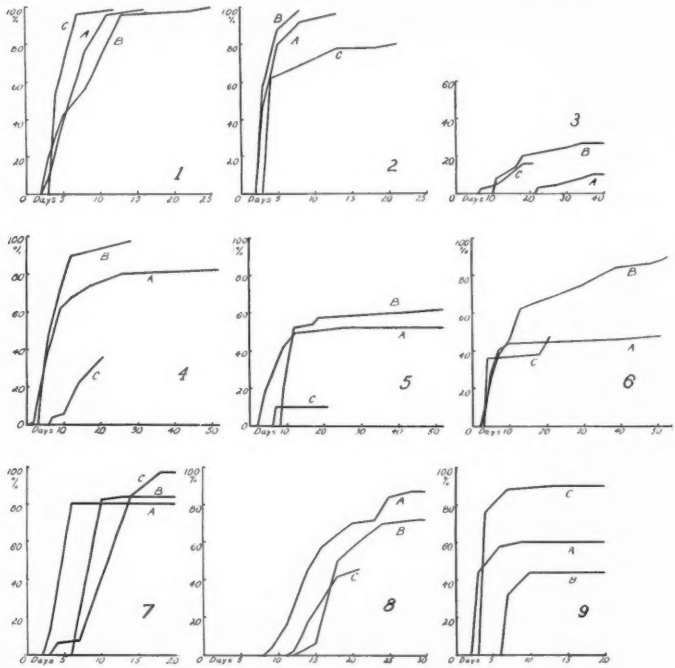
TABLE III

EFFECT OF ALTERNATE MOISTENING AND DRYING ON PERCENTAGE OF GERMINATION

Species	Percentage germination			
	Increase with rapid drying	Increase with slow drying	Decrease with rapid drying	Decrease with slow drying
Bromus anomalus	.....	2	14	.....
B. polyanthus	2	0	.....	0
Poa interior	2	.....	.....	16
P. secunda no. 1	4	.....	.....	14
P. secunda no. 2	4	.....	.....	10
Stipa columbiana	.....	.....	4	4
S. lettermani	16	16	.....	.....
Achillea lanulosa	16	.....	.....	38
Androsace diffusa	10	.....	.....	40
Artemisia incompta	.....	26	.....	.....
Chenopodium album	.....	.....	2	10
Geranium viscosissimum	.....	.....	5	15
Lepidium densiflorum	42	4	.....	.....
Lupinus parviflorus	.....	5	25	.....
Pentstemon rydbergii	12	.....	.....	2
Plantago tweedyi	4	18	.....	.....
Pseudocymopterus montanus	.....	.....	14	24
Rudbeckia occidentalis	.....	30	16	.....
Rumex mexicanus	30	.....	.....	22
Chrysothamnus lanceolatus	.....	.....	22	2

The influence of alternate moistening and drying as shown by increase or decrease in percentage of germination is given in table III. Since the test in which the seeds were dried slowly (*C*) extended over a period of 21 days only, and tests *A* and *B* extended over 51 to 53 days, the final results of test *C* are not comparable with the other two except in six species, *Poa interior*, *P. secunda* no. 2, *Chenopodium*, *Geranium*, *Plantago*, and *Rudbeckia*, in which germination occurred within 21 days. However, the effect of alternating moisture

conditions on the percentage and the rate of germination during the period of test *C* and the early period of tests *A* and *B* may be compared. The increase or decrease in percentage of germination of the



FIGS. 1–9.—Graphs showing rate and percentage of seed germination: *A*, given continuous moisture; *B*, subjected to alternating moisture conditions in which drying was rapid; *C*, subjected to alternating moisture conditions with slow drying. Fig. 1, *Bromus polyanthus;* fig. 2, *Poa interior;* fig. 3, *Stipa lettermani;* fig. 4, *Achillea lanulosa;* fig. 5, *Androsace diffusa;* fig. 6, *Lepidium densiflorum;* fig. 7, *Plantago tweedyi;* fig. 8, *Pseudocymopterus montanus;* fig. 9, *Rudbeckia occidentalis.*

seeds from test *C* is based, therefore, on the germination of seeds which were kept moist continuously (test *A*) for a period of 21 days, and not on the final percentage of germination. Decreased germination was accompanied by a delay and increased germination by hastening, except with *Poa secunda* no. 1, *Androsace*, *Plantago*, *Rudbeckia*, and *Rumex*, in which a delay at first was followed later

by increased germination. In *Rudbeckia* and *Rumex* the retardation was slight. Differences of 5 per cent or less in germination results are not considered of importance. Figures 1 to 9 show the percentage and rate of germination in each of nine species representative of the different ways in which the seeds were affected.

TABLE IV

GERMINATION OF SEEDS FROM TEST B AFTER PERIOD OF ALTERNATE MOISTENING AND DRYING

Species	Percentage germination		
	During period of alternate moistening and drying	After period of alternate moistening and drying	Total
Grasses			
Agropyron pauciflorum	2	20	22
Hordeum nodosum	0	74	74
Poa secunda no. 1	68	2	70
P. secunda no. 2	20	6	26
Herbs other than grasses			
Achillea lanulosa	98	2	100
Collomia linearis	0	62	62
Frasera speciosa	0	5	5
Gilia pulchella	0	8	8
Lepidium densiflorum	90	2	92
Lupinus parviflorus	70	5	75
Pentstemon rydbergii	16	6	22
P. subglaber	2	12	14
Plantago tweedyi	84	2	86
Polemonium foliosissimum	0	20	20
Polygonum douglasii	0	5	5
Rumex mexicanus	52	2	54
Vicia americana	5	30	35
Woody species			
Chrysothamnus lanceolatus	64	2	66
Ribes inebrians	0	2	2

GERMINATION OF SEEDS AFTER ALTERNATE MOISTENING AND DRYING

Some of the seeds which failed to germinate when subjected to alternating moisture conditions with rapid drying (test *B*) germinated when placed in other conditions following the period of alternate moistening and drying (table IV).

TABLE V

GERMINATION OF SEEDS SUBJECTED TO LONG PERIOD OF DRYING

SPECIES	NO. SEEDS USED	FIRST GERMINATING CONDITIONS	PERCENTAGE GERMINATION	NO. SEEDS DRIED	GERMINATING CONDITIONS AFTER DRYING	PERCENTAGE DRIED SEEDS GERMINATING
Agropyron	50	Alternating moisture 21 days	4	48	Cold, then warm	41
Hordeum	50	23° to 25° 35 "	4	42	Alt. temp.	97
Hordeum	50	Alternating moisture 21 "	0	49	Alt. temp.	96
Melica	50	−1.5° to −2.5° 10 " 23° " 25° 25 "	0	49	Cold	94
Melica	50	19° " 24° 35 "	0	49	Cold	84
Melica	50	22° " 29° 74 "	0	49	Cold	92
Androsace	50	3° " 7° 96 " 17° " 21° 51 "	0	46	Alt. temp.	20
Castilleja	50	3° " 7° 96 " 17° " 21° 51 "	2	45	Cold, then alt. temp.	4
Castilleja	50	22° " 29° 74 "	0	36	Cold, then alt. temp.	0
Chenopodium	50	22° " 29° 74 "	12	41	Cold, then alt. temp.	2
Chenopodium	50	19° " 24° 35 "	8	42	Cold, then alt. temp.	7
Chenopodium	50	3° " 7° 96 " 17° " 21° 51 "	18	41	Cold, then warm	0
Collomia	50	22° " 29° 74 "	0	41	Cold, then alt. temp.	7
Collomia	50	3° " 7° 96 " 17° " 21° 51 "	40	29	Cold, then alt. temp.	13
Delphinium	50	3° " 7° 96 " 17° " 21° 51 "	2	48	Cold, then warm	0
Delphinium	50	Alternating moisture 53 "	0	27	Cold, then alt. temp.	0
Erythronium	20	22° to 29° 74 "	0	19	Cold, then alt. temp.	0
Frasera	20	22° " 29° 74 "	0	16	Cold, then alt. temp.	25
Gilia	50	22° " 29° 74 "	0	23	Cold, then alt. temp.	9
Gilia	25	3° " 7° 96 " 17° " 21° 51 "	0	24	Cold, then warm	12
Lupinus	20	3° " 7° 96 " 17° " 21° 51 "	90	2	Cold, then warm	50
Moldavica	50	22° " 29° 74 "	2	48	Alt. temp.	0
Moldavica	50	18° " 22°204 "	2	48	Alt. temp.	0
Moldavica	50	3° " 7° 96 " 17° " 21° 51 "	2	48	Cold, then warm	0
Orthocarpus	50	19° " 24° 35 "	0	49	Cold, then alt. temp.	0
Orthocarpus	50	3° " 7° 96 " 17° " 21° 51 "	4	46	Cold	0

TABLE V—*Continued*

Species	No. seeds used	First germinating conditions	Percentage germination	No. seeds dried	Germinating conditions after drying	Percentage dried seeds germinating
Pentstemon ryd-bergii	50	3° to 17° 7° to 21° 96 days	14	43	Cold, then alt. temp.	19
P. subglaber	50	Alternating moisture 53 "	2	46	Cold, then alt. temp.	13
P. subglaber	50	3° to 17° 7° to 21° 96 "	6	47	Cold, then warm	0
Polemonium	50	22° " 29° 74 "	2	46	Cold, then alt. temp.	26
Polemonium	50	3° " 17° 7° to 21° 96 "	4	46	Cold, then warm	9
Rumex	50	Alternating moisture 21 "	0	50	Alt. temp.	86
Sophia	50	3° to 17° 7° to 21° 96 "	0	46	Cold, then warm	2
Vicia	25	3° " 17° 7° to 21° 96 "	8	22	Seed coat filed; cold then warm	100
Vicia	25	23° " 25° 26 "	0	16	Cold, then alt. temp.	0
Viola	50	Alternating moisture 21 "	0	37	Cold, then alt. temp.	0
Ribes inebrians	50	23° to 25° 35 "	0	45	Alt. temp.	4
Ribes inebrians	25	3° " 17° 7° to 21° 97 "	0	24	Cold, then alt. temp.	17
Ribes inebrians	50	Alternating moisture 53 "	0	36	Cold, then alt. temp.	3
Ribes inebrians	50	22° to 29° 74 "	0	44	Cold, then alt. temp.	0
R. montigenum	50	23° " 25° 35 "	0	33	Alt. temp.	0
R. montigenum	25	3° " 17° 7° to 21° 97 "	0	24	Cold, then alt. temp.	17
Sambucus	50	23° " 25° 35 "	0	44	Alt. temp.	0
Sambucus	50	3° " 17° 7° to 21° 97 "	0	50	Cold, then warm	0
Sambucus	50	22° " 29° 74 "	0	48	Cold, then alt. temp.	0
Symphoricarpos	50	23° " 25° 35 "	0	43	Alt. temp.	0
Symphoricarpos	50	Alternating moisture 21 "	0	42	Cold, then alt. temp.	0
Symphoricarpos	50	18° to 22° 204 days	0	32	Cold, then alt. temp.	3

Of the five species which were kept from test *C*, the *Agropyron* seeds gave 40 per cent, *Hordeum* 94 per cent, and *Rumex* 86 per cent additional germination. *Symphoricarpos* and *Viola* seeds did not germinate under the new conditions.

Germination after long period of drying

Percentages of germination obtained with the seeds which had been dried for 105 days after having been in moist conditions for varying lengths of time are shown in table V. Nineteen of the 25 species and 28 of the 48 samples used gave varying percentages of germination.

Discussion

The effect of alternate moistening and drying varied with the different species. In a few instances the effect of drying on the percentage of germination was negligible. Germination in some species was hastened and the percentage of germination increased by alternate moistening and drying, while with others germination was delayed and the percentage of germination decreased. Rapid and slow drying varied in their effect on germination in a number of the species studied. The behavior of the seeds depends upon the particular species, the rate of drying, and the time when drying takes place.

In general, the seeds of the species which germinated at room temperatures may be placed in five groups: (1) seeds in which alternate moistening and drying had no effect on percentage of germination; (2) seeds in which the percentage of germination was increased under conditions of alternate moistening and drying; (3) seeds in which the percentage of germination was decreased under conditions of alternate moistening and drying; (4) seeds in which germination was increased with rapid drying and decreased with slow drying; and (5) seeds in which germination was decreased with rapid drying and increased with slow drying.

While alternate moistening and drying had no effect on percentage of germination of *Bromus polyanthus*, rapid drying slightly delayed and slow drying hastened its germination (fig. 1).

Seeds giving increased germination have been noted in table III. The amount of increase usually varied with rapid drying and slow drying. However, both types of drying gave the same increase in percentage of germination in *Stipa lettermani*. The most pronounced effect of increase in germination was found in *Lepidium* (fig. 6) and *Rumex*, with rapid drying, and in *Artemisia* and *Rudbeckia* with slow

drying. The highest percentage of germination obtained in *Androsace* was with seeds which had been subjected to alternating moisture conditions with rapid drying.

With some seeds germination continued during the period of drying. In test *B* the seeds of *Poa interior* (fig. 2) began to germinate before the petri dishes were opened to dry, and continued to germinate during the period of drying. The seeds thus subjected to drying gave a slightly higher percentage of germination in a shorter period of time than the seeds which had continuous moisture. The behavior of the two lots of *Poa secunda* seeds was similar to, but lower in percentage, than *P. interior*. Germination also continued during the slow drying of the seeds of *Bromus anomalus*, *B. polyanthus*, *Artemisia*, and *Rudbeckia* (fig. 9).

In several species the percentage of germination secured with continuous moisture was lower at the temperatures used than the maximum germination obtained under other conditions (table I). Although temperature conditions were not favorable, percentages of germination approaching the maximum were obtained in *Achillea* and *Lepidium* under alternating moisture conditions with rapid drying, and in *Artemisia*, *Plantago*, and *Rudbeckia* with slow drying. In these species the effect of temperature was overcome by drying. In *Stipa lettermani* and *Rumex mexicanus* the effect of temperature was overcome by drying only in part. Under conditions of alternating temperatures *Rumex* seeds gave 100 per cent germination in 12 days. The effect of drying on *Rumex* was slowly to bring about the changes in the seed which were quickly induced by alternating temperatures. The foregoing results suggest that the effect of drying in these seeds is associated, at least in part, with the problem of oxygen supply, as CROCKER (3) found for *Xanthium*.

Drying decreased the percentage of germination in the seeds of several species. The amount of injury varied with the species and was influenced by the time and rate of drying. *Pseudocymopterus* (fig. 8) illustrates this effect. Slow drying of *Geranium* decreased germination slightly by hardening the seed coat of the few seeds which did not germinate quickly. Rapid alternations of moistening and drying only retarded the process of germination. The behavior

of *Geranium* agrees with the conclusions of DUVEL, who found that the retardation in germination after dry storage was caused by changes in the seed coat.

The knowledge regarding the nature of the changes taking place in the seeds of each particular species is far from complete. Drying may have produced changes in the seed coat or embryo, or in both seed coat and embryo. JOSEPH (19) observed that drying parsnip seeds during the storage period affected the embryo and not the seed coat. KIDD and WEST (21) concluded that the redrying of *Brassica alba* seeds overcame secondary dormancy, but DAVIS (8) could not secure any benefit from redrying *Ambrosia trifida* seeds in which secondary dormancy had been induced. JONES (18) observed that desiccation of after-ripened *Acer saccharum* seeds reduced seedling vigor, and that if desiccation continued the embryos failed to germinate. FLEMION (12) found that drying partially or entirely after-ripened seeds of *Sorbus aucuparia* developed a secondary dormancy which required longer to overcome than the original period of dormancy. She discovered too, that while the chief cause of dormancy was in the embryo, the seed coat also contributed to delay after the embryo had after-ripened. The effect of drying therefore differs with the different types of seeds.

IMPORTANCE OF TIME AT WHICH DRYING OCCURS

The time at which drying occurs is very important with some seeds. The time of drying in relation to the stage of development of the germinating seed was probably a factor in producing the results shown in table III, where rapid and slow drying gave opposite results. The effect of decreasing resistance to drying is seen in the slow drying of *Poa interior*, *P. secunda*, *Achillea* (fig. 4), and *Androsace* (fig. 5), and in both types of drying of *Chrysothamnus* and *Pseudocymopterus*. With rapid drying, which began late, *Bromus anomalus*, *Lupinus*, and *Rudbeckia* were injured. *Poa secunda* seeds with low viability did not withstand drying that started early in the germinative process. PFEFFER (27) calls attention to the fact that while resistant seeds can withstand drying and remoistening, seedlings are killed by drying, and resistance to drying decreases during germination.

Hastening of germination occurred in some seeds after slow drying (fig. 7) and in others after rapid drying (figs. 4, 8). In *Pseudocymopterus*, germination was hastened after the second period of rapid drying, although further drying decreased the percentage of germination. Such hastening and acceleration in germination of *Brassica alba* seeds after drying, as found by KIDD (**20**) and KIDD and WEST (**21**), may be due to the stage of development attained by the embryo while in a moist condition previous to drying, rather than to the influence of drying.

INFLUENCE OF CONDITIONS PREVIOUS TO DRYING

The influence of conditions previous to drying may be seen in the germination of some of the seeds which were dried for 105 days (table V). The three sets of *Melica* seeds were subjected to identical conditions during and after the period of drying. After drying and remoistening, followed by cold temperatures of 0° to 4° C. for 84 days, the seeds gave 94, 84, and 92 per cent germination respectively. Those seeds which had been placed at 19° to 24° C. for 35 days had not made as much development previous to drying as had the seeds which had been subjected to − 1.5° to − 2.5° C. for a part of the moist period, or those which had a longer period of moisture at the warmer temperatures. Although the percentage of germination was low, *Collomia* and *Ribes inebrians* also show the influence of conditions previous to drying. Here, as in *Melica*, the seeds which had been held at low temperatures previous to drying gave higher percentages of germination than those which had been given warm temperatures. Such results indicate that drying during the period of after-ripening had little, if any, effect upon the germination of seeds of *Melica*, *Collomia*, and *Ribes inebrians*.

EFFECT OF ALTERNATE MOISTENING AND DRYING AS SHOWN BY LATER TEST FOR VIABILITY

The effect of alternate moistening and drying was not shown on some seeds until after the period of alternating moisture conditions, when the seeds were placed in other conditions for germination. No germination was obtained during the time *Hordeum nodosum* and *Collomia linearis* seeds were subjected to alternating moisture con-

ditions with rapid drying. Apparently they were not harmed by such conditions and derived some benefit. In the test for viability following the period of alternate moistening and drying (table IV), *Hordeum* seeds from test *B* gave 74 per cent germination. While this was below the maximum obtained (table I), it was 50 per cent more than that secured from seeds under similar germinating conditions which had not previously been subjected to alternate moistening and drying. *Hordeum* seeds from test *C* which were dried for 105 days gave 94 per cent germination with alternating temperatures. (The percentage here is based on the original number of seeds and not on the number of seeds dried.) *Collomia* seeds which had been subjected to alternate moistening and drying in test *B* later gave the maximum germination obtained, 62 per cent, in a shorter period of time than seeds which had not been so treated.

When placed in new conditions for germination after the period of alternate moistening and drying, *Agropyron* and *Polemonium* each gave 20 per cent and *Pentstemon subglaber* gave 12 per cent additional germination. A few species gave less than 10 per cent germination.

Some seeds require a longer period for germination than was supplied to them in the test for viability following the period of alternate moistening and drying.

The effect of alternate moistening and drying on hardcoated seeds may be seen in *Lupinus parviflorus* and *Vicia americana*. In tests *A*, *B*, and *C*, the seed coats of both species were kept intact. Rapid alternations of moistening and drying retarded and decreased the germination of *Lupinus* somewhat, but had little effect on *Vicia*. Seeds of *Vicia* which were given continuous moisture gave no germination and seeds which were subjected to alternating moisture conditions gave only 5 per cent germination with either rapid or slow drying. After the period of alternate moistening and drying, the coats of the *Vicia* seeds which had not germinated in test *B* were broken by filing and the seeds again supplied with moisture. With this treatment 30 per cent additional germination was obtained, thus giving 35 per cent total germination for this set of seeds (table IV). At the same time, seeds which had not been subjected to alternating moisture conditions but in which the coats were broken by filing, gave 40 per cent germination.

Failure of seeds to germinate when placed in other conditions after 51 to 53 days of alternating moisture conditions, therefore, may have been due to injury from previous drying; to secondary dormancy produced by the drying, temperature, or moisture conditions; to lack of suitable germinating conditions; or in a few instances to lack of viability. Some seeds were injured by the alternate moistening and drying and others withstood repeated drying with little or no injury. Some of the *Poa secunda* seeds were not viable. Seeds from the second area had much lower viability than seeds from the first area. *Polygonum* seeds which failed to germinate and some seeds of *Pentstemon rydbergii*, *Acer grandidentatum*, and *Sambucus microbotrys* were found to be undeveloped. A few seeds molded during the period of testing, but many which failed to germinate still appeared sound. Since these seeds did not germinate with continuous moisture at room temperatures (22° to 29° C.) within the time employed, other conditions will have to be used for them before the effect of alternating moisture conditions can be determined. The seeds of a number of the species contain rudimentary embryos or embryos which require a long period of after-ripening before germination occurs.

Effect of long period of drying on germination behavior

Seeds which were dried for 105 days after a period of moistening also varied in their responses to germinating conditions (table V). Some may not have had suitable temperatures for germination, or the period may not have been long enough. One or more samples of each of four species, *Melica*, *Pentstemon rydbergii*, *Vicia*, and *Ribes montigenum*, gave higher percentages of germination after the long period of drying than the maximum germination obtained from seeds which were not so dried. Dried seeds of *R. inebrians* gave next to the highest germination obtained for the species. The behavior of *Melica* and *R. inebrians* seeds has been discussed in connection with the influence of conditions previous to drying. Of the two samples of *Vicia* seeds which were dried, those in which the seed coat had been broken by filing before they were remoistened gave 100 per cent germination. Those with coats intact gave no germination until later; when the coats were broken by filing, they also gave 100 per

cent germination. The long period of drying had no harmful effect on these seeds. The behavior of *V. americana* corresponds to the findings of MUNERATI and ZAPPAROLI for *V. segetalis*.

Some of the dried *Pentstemon rydbergii* seeds which failed to germinate were undeveloped, but 19 per cent germinated when remoistened. This was equal to 16 per cent of the original lot from which the dried seeds were taken. Previous to drying, 14 per cent had germinated, thus giving a total of 30 per cent for this lot of seeds, which was the maximum obtained for *P. rydbergii*. Thus germination was increased by drying the ungerminated seeds.

Little or no injury was shown by seeds of *Hordeum* and *Polemonium*. *Hordeum* seeds which had not been dried gave 100 per cent germination, and seeds which had been dried gave 96 to 97 per cent germination when subjected to alternating temperatures. *Polemonium* seeds which had been dried for 105 days gave 26 per cent germination while the maximum obtained in other tests was only 30 per cent.

Decreased germination of *Rumex* and *Orthocarpus* may have been due to injury or to secondary dormancy produced by drying, or by conditions which prevailed during the previous moist period. With alternating temperatures, *Rumex* seeds which had not been dried gave 100 per cent germination, but the seeds which had been dried after the period of alternating moisture conditions with slow drying gave only 86 per cent germination. *Orthocarpus* seeds gave 28 per cent germination when subjected to 0° to 4° C. for 87 days, but seeds which had been dried gave no germination although subjected to a temperature of 0° to 3° C. for a period of 91 days.

Lower percentages of germination than the maximum obtained with seeds which were not dried were obtained from the dried seeds of *Agropyron*, *Androsace*, *Chenopodium*, *Collomia*, *Frasera*, and *Gilia*. Besides *Orthocarpus*, which has been discussed, no germination occurred in the dried seeds of five other species, *Delphinium*, *Erythronium*, *Moldavica*, *Viola*, and *Sambucus*. Only one or two of the dried seeds of each species germinated in *Castilleja*, *Sophia*, and *Symphoricarpos*. Since several of these seeds have rudimentary embryos or embryos which require after-ripening, failure to germinate may have

been due to lack of suitable conditions for germination, or to secondary dormancy caused by drying rather than to injury or lack of viability.

Effect of drying on germination

The effect of drying on germination behavior is determined by the effect of drying on the seed itself. Drying seeds after they have absorbed water may affect them in one or more of the following ways:

1. Lack of sufficient moisture may arrest the development or growth of the embryo which precedes germination, until sufficient moisture is again supplied. When this is supplied development and growth proceed without injury and germination is only delayed.

2. Drying of the embryo at a critical stage in its growth, for example, when it is well developed just previous to or during the early stages of germination, when cell division is rapidly taking place, may injure it beyond the point of recovery and thus prevent further growth and germination when moisture is again supplied, as noted by Kidd, Kidd and West, and Pfeffer. This results in decreased germination.

3. Drying may bring about changes in the seed coat, making it more permeable to water or oxygen, or it may lessen the mechanical resistance of the coat to the developing embryo as found by Ludwig and Crocker (**3, 4, 6**). Such changes hasten and increase germination while the reverse of these changes in the seed coat may produce secondary dormancy, and thus delay or prevent germination (**9**).

4. Drying may bring about changes in the embryo which lower the vitality of the seed, decreasing germination, as found in the parsnip by Joseph and in the sugar maple by Jones (**18**); or it may produce secondary dormancy which delays and decreases germination until dormancy is again overcome, as in *Sorbus aucuparia* (**12**). Drying may bring about other changes in the embryo which hasten the development and germination of dormant seeds when moisture is again supplied. The behavior of some of the seeds used in this investigation indicates that the effect of drying may be on the embryo rather than on the seed coat. Further study is needed to determine definitely which part of the seed is affected.

The rate at which drying occurs and the time of drying often

determine whether it is harmful, beneficial, or negligible in its effect on germination. The amount of benefit or injury by alternate moistening and drying is difficult to determine, in some cases, until more is known about the normal germination behavior of the seeds. They vary considerably in their ability to withstand drying. Conditions which inhibit or delay the germination of seeds of one species stimulate and increase germination in another, and have no effect upon a third. The factors which bring this about can be determined only by further study of the individual species.

Summary

1. The seeds used in this study comprise 9 grass, 26 weed, and 7 woody species of Utah range plants. Twenty-three of the 42 species studied gave little or no germination at temperatures of 22° to 29° C. Other conditions must be used for them before the effect of alternate moistening and drying can satisfactorily be determined.

2. The effect of alternate moistening and drying on the germination of seeds varies with the individual species. Of the 19 species which germinated at 22° to 29° C., alternate moistening and drying had little effect upon the germination of *Bromus polyanthus*. It increased the germination of *Stipa lettermani*, *Artemisia incompta*, *Lepidium densiflorum*, and *Plantago tweedyi*. It decreased the germination of *Geranium viscosissimum*, *Pseudocymopterus montanus*, *Chrysothamnus lanceolatus*, and *Stipa columbiana* (slightly).

3. Rapid drying had little effect upon the germination of *Poa interior*, *P. secunda*, and *Chenopodium album*, but increased the germination of *Achillea lanulosa*, *Androsace diffusa*, *Pentstemon rydbergii*, and *Rumex mexicanus;* in all of these species slow drying decreased the percentage of germination. Rapid drying decreased the germination of *Bromus anomalus*, *Lupinus parviflorus*, and *Rudbeckia occidentalis*, while slow drying increased the germination of *Rudbeckia* but had little effect upon *Bromus anomalus* and *Lupinus*.

4. Increased germination was usually accompanied by a hastening of the germinative process and decreased germination was accompanied by a retardation. In five species, however, *Poa secunda* no. 1, *Androsace*, *Plantago*, *Rudbeckia*, and *Rumex*, retardation was followed by increased germination.

5. Some seeds after they have absorbed water will withstand short periods of alternate drying and moistening, and some will withstand a long period of drying without injury. The time at which drying begins in relation to the stage of development of the germinating seed is a very important factor in determining whether drying is harmful or beneficial. The effect of drying is also influenced by the rate at which drying takes place.

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GREAT BASIN BRANCH STATION
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RETARDED GERMINATION IN THE SEED OF HYPERICUM PERFORATUM CAUSED BY CALCIUM[1]

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(WITH SIX FIGURES)

Introduction

Hypericum perforatum, locally known as Klamath weed, is a serious pest in certain northern California range lands (**6**), where it has almost completely replaced the range forage. Studies of control methods, including both chemicals and range management, have been under way since 1930.

Early in the studies of conditions of seed germination, it was observed that germination takes place much more rapidly in distilled than in tap water, which resembles the soil solutions in which the seeds usually germinate.

Methods

Seeds of *Hypericum perforatum* germinate as well, if not more readily, when submerged in water as when grown on moist canton flannel.

A liquid germination medium has, for these experiments, certain distinct advantages. Petri dishes containing 25 cc. of liquid can easily be handled without danger of spilling, and since this amount of water or solution of salts is adequate for the tests, it was used in all cases. All tests were run in duplicate, and many in triplicate. Since the seeds are very small, 200 could be used in each dish, making 400 for each test. With this number the error of random sampling is reduced to a minimum.

This species does not germinate readily in the absence of light (**3**) unless nitrate is present in the germination medium (**2**). Germination in darkness proved considerably lower than in light. In both light and darkness, however, germination was far better in distilled than in tap water. For the purpose of these experiments, therefore,

[1] This investigation was conducted while the writer was at the University of California, Davis, California.

control of light appeared unnecessary provided the various lots of each experiment were subjected to the same light fluctuations. The petri dishes were grouped on a laboratory table where all were apparently equally exposed to the diffused light of the room.

Comparative tests were always run simultaneously so that all were subjected to the same temperature fluctuations.

TABLE I

PERCENTAGE GERMINATION OF SEEDS IN TAP AND DISTILLED WATER

	PERCENTAGE GERMINATION			
	END OF 4 DAYS		END OF 7 DAYS	
	TAP WATER	DISTILLED WATER	TAP WATER	DISTILLED WATER
	2.5	58.0	48.2	88.5
	1.7	78.5	44.2	96.0
	4.0	62.0	44.2	93.2
	1.5	45.2	55.0	88.5
	2.5	46.5	41.5	92.0
	3.5	58.0	49.5	93.0
	2.0	61.5	50.5	88.7
	2.0	54.0	47.5	91.7
	4.0	69.5	40.0	92.5
	2.0	47.5	29.5	93.5
Mean..	2.6	58.1	45.0	91.8

Experimental results

DIFFERENCES IN GERMINATION IN TAP AND DISTILLED WATER.—The data of table I are taken from the tap and distilled-water check lots from each of ten experiments. Each figure in the table is based on at least duplicate samples of 200 seeds each.

Figure 1 presents additional data, showing the rate of germination from the fourth to the ninth days inclusive. The points on each curve are based on germination data from 1200 or more seeds.

According to these data the effect of the tap water is mostly one of retardation. Figure 1 shows that the germination in tap water on the ninth day is only slightly higher than that attained in distilled

water on the fourth day. Presumably the germination in tap water might eventually have become as high as that in distilled had the tests been continued longer. Although germination was greatly retarded, there was little evidence that the tap water was toxic; the seedlings produced were healthy and vigorous.

The seeds sink much more readily after a few hours' soaking in distilled than in tap water, and they change color in tap water. The

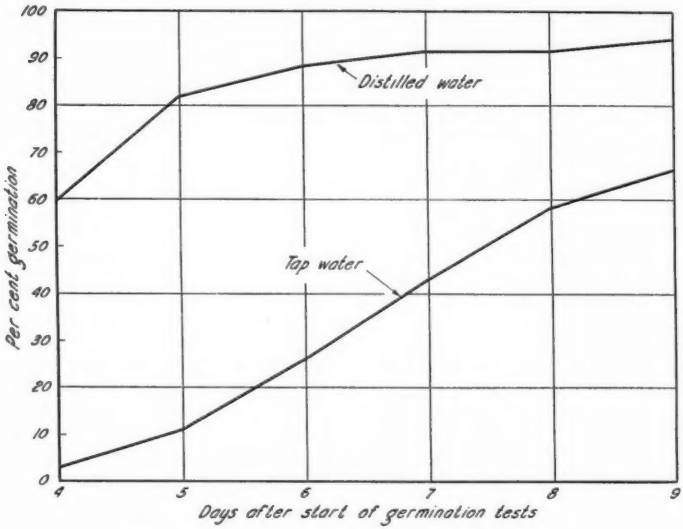

FIG. 1.—Rate of germination in tap and distilled water

dry seed, when mature, is brown, and when placed in distilled water it retains this color. In tap water, however, the seeds become jet black after a few hours of soaking.

Since a striking difference occurred in rate of germination in the two media, the effect of mixing the two was studied. The amount of tap water was varied by increments of 10 per cent from 0 to 100 per cent. As germination in all these mixtures was practically the same as in the tap-water check lots, evidently the critical concentration lay below 10 per cent tap water. A new series (fig. 2) was accordingly prepared with dilutions of 1, 3, 5, 7, 9, and 10 per cent tap water.

A mixture containing only 1 per cent of tap water distinctly retarded germination; a 3 per cent solution caused even greater retardation; and 5 per cent reduced the germination at the fourth day to about the same value as in pure tap water. At the end of seven days all lots showed much improvement, but especially the lots containing least tap water. No lot equaled the distilled water check at that time.

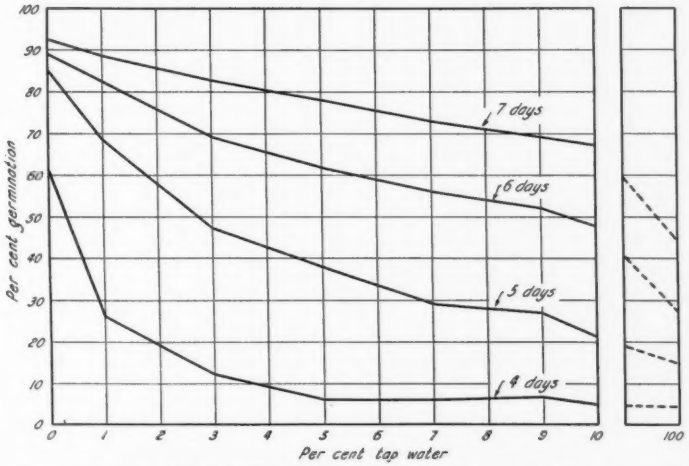

FIG. 2.—Germination in distilled water, tap water, and mixtures of the two containing 90 per cent or more distilled water.

In other experiments seeds were first placed in tap water and later changed to distilled. An extremely short immersion in tap water at the beginning of the experiment retarded germination materially (table II). Comparison of lots 1 and 2 of table II shows that one hour in tap water produced a retardation from which the lot did not fully recover until the seventh day of the test. Longer initial treatments with tap water produced even more pronounced delays.

POSSIBLE CAUSES OF LOW GERMINATION IN TAP WATER.—Attempts to explain the cause of these different germination responses suggested two lines of investigation: first, the effect on germination of various constituents of tap water; second, the effect of the pH of the medium. The tap water has a pH of 8.4 or higher, while the dis-

tilled water is slightly less than 6. This difference might account for the difference in rate of germination observed. On the other hand, the tap water is fairly high in magnesium, sodium, and bicarbonate ions, and conceivably these or other constituents might interfere with germination. Data are presented dealing with both these assumptions.

TABLE II

RETARDING EFFECT ON GERMINATION CAUSED BY SHORT PERIODS IN TAP WATER

TREATMENT	PERCENTAGE GERMINATION			
	4TH DAY	5TH DAY	6TH DAY	7TH DAY
Distilled water check	17	59	78	81
Tap H_2O 1 hour, then distilled H_2O	5	39	72	82
" " 2 hours " " "	3	33	68	79
" " 3 " " " "	4	32	67	83
" " 5 " " " "	3	35	69	82
" " 7 " " " "	4	28	66	82
" " 13 " " " "	2	24	58	73
" " 24 " " " "	3	23	55	78
" " 36 " " " "	4	26	59	78
" " 48 " " " "	3	20	58	80
" " 72 " " " "	1	12	39	64
Tap H_2O check	1	8	24	37

EFFECT ON GERMINATION OF VARIOUS IONIC COMPONENTS OF TAP WATER.—The domestic water supply of the University Farm at Davis, California, is obtained from a deep well. The ions most abundant in it are present in the following approximate concentrations (milliequivalents per liter):

$Na+$ . . . . . -2.3		$CO_3^=$ . . . . . -0.30
$K+$ . . . . . -0.0		HCO_3^- . . . . . -6.08
$Ca++$ . . . . . -1.4	Cl^- . . . . . -0.38	$NO_2^- + NO_3^-$ . . . -0.05
$Mg++$ . . . . . -3.7	$SO^=$ . . . . . -0.63	$H_2AlO_3^-$ . . . . . -0.11

To study the effect of these various ions on germination, tests were run in solutions of various salts of Na, Ca, and Mg. In these solutions the concentration of each positive ion was made approximately equal to its concentration in tap water. Nitrate, sulphate, phosphate, chloride, and bicarbonate were the anions used. The results

indicated little difference between the sodium and magnesium salts; but the calcium salts gave consistently low results.

Inasmuch as single salts are sometimes toxic, solutions were then made up containing two salts each (table III). In those solutions which contained both sodium and magnesium, germination was high. When either of these two was used with calcium, however, the rate of germination was sharply reduced, although not to so low a value as that of tap water. These results confirm the observations made on germination tests run in single salt solutions, and indicate that primarily calcium causes the retardation.

TABLE III

EFFECT ON GERMINATION OF VARIOUS IONIC CONSTITUENTS OF TAP WATER

CONTENT OF SOLUTION	PERCENTAGE GERMINATION			
	4 DAYS	5 DAYS	6 DAYS	7 DAYS
$MgSO_4$*+$NaCl_2$	22	58	80	86
$CaSO_4$ +NaCl	9	34	58	75
$MgSO_2$ +$CaCl_2$	7	33	63	75
Tap water	1	5	13	29
Distilled water	34	69	81	88

* In each solution, each positive ion used was present in a concentration of three milli-equivalents per liter.

Since calcium salts retarded germination, their effect in different concentrations was studied. Calcium chloride was prepared in concentrations in which the calcium ranged from 0.02 to 200 times its concentration in tap water. Figure 3 shows the germination results from these solutions and from the tap and distilled water checks. It will be seen that the distilled-water check slightly surpasses even the most dilute calcium chloride solution used. The tap-water check, on the other hand, is slightly higher than a solution containing double the amount of calcium but considerably less than one containing 0.2 times as much calcium. These results show a close relationship between rate of germination and amount of calcium present in solution. They also check with the results obtained by diluting tap water with distilled (fig. 2).

As already shown, sodium salts have little influence on rate of germination. A pure sodium chloride solution containing 100 milliequivalents per liter gave better results than one of calcium chloride containing only 2.8 milliequivalents per liter. A series of mixtures of

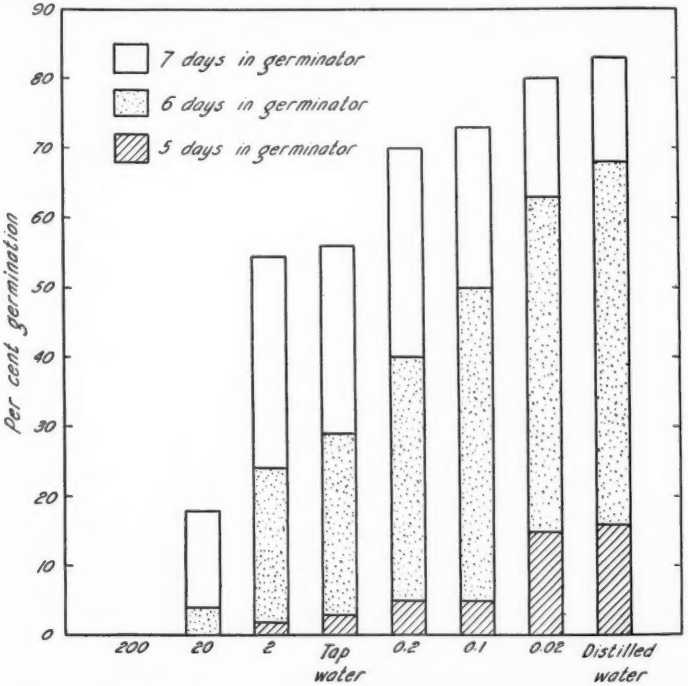

FIG. 3.—Germination in tap water, distilled water, and various concentrations of calcium chloride. Concentration of calcium chloride expressed in multiples of amount present in tap water.

the two salts was then prepared, in all of which the calcium chloride concentration was 2.8 milliequivalents per liter. The sodium chloride content was varied from 1 to 100 milliequivalents per liter. In all these the germination was as low as in pure calcium chloride of 2.8 milliequivalent concentration, indicating that this particular effect of calcium is not prevented by antagonism of sodium.

RELATION OF pH OF SOLUTION TO RATE OF GERMINATION.—The pH of the tap and distilled water mixtures previously discussed (fig. 2)

was not determined. A series (ranging from 0 to 100 per cent tap water) prepared later, however, showed that pH decreased with decreased concentration of tap water (fig. 4). Although data presented in figure 3 indicate that the concentration of calcium in these tap-water mixtures probably controls the germination results observed (figs. 2, 4), they do not prove that the pH variations are not in part responsible.

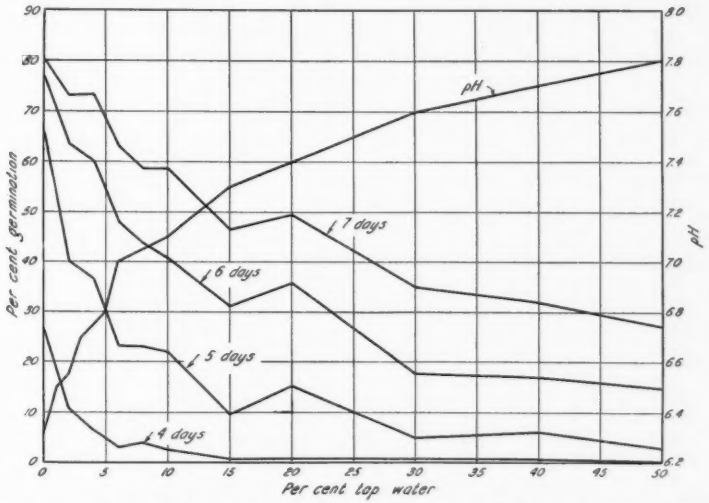

FIG. 4.—Relation of pH of various mixtures of tap and distilled water to germination in these mixtures.

A series of buffers was prepared containing the primary and secondary phosphates of sodium in total concentration equivalent to $\frac{M}{300}$. In this dilution these buffers gave a pH range from about 6 to 7.1, which covers the region in figure 4 where greatest differences in germination occur. The data (fig. 5) show that these variations in pH result in no significant difference in rate of germination. The buffers had no deleterious effect on the seeds; something other than pH causes the irregularities of germination in tap water.

This experiment was repeated with one modification. The buffer solutions were made up as before, but each solution was divided into two parts. One part was used as it was; to the other was added cal-

cium chloride at the rate of 2.3 milliequivalents of calcium per liter. In neither series was any significant difference found among the lots that could be attributed to differences in pH. The average germination for all lots in each series and those of the checks in tap and distilled water are plotted in figure 6.

While the germinations in phosphate buffers and in distilled water

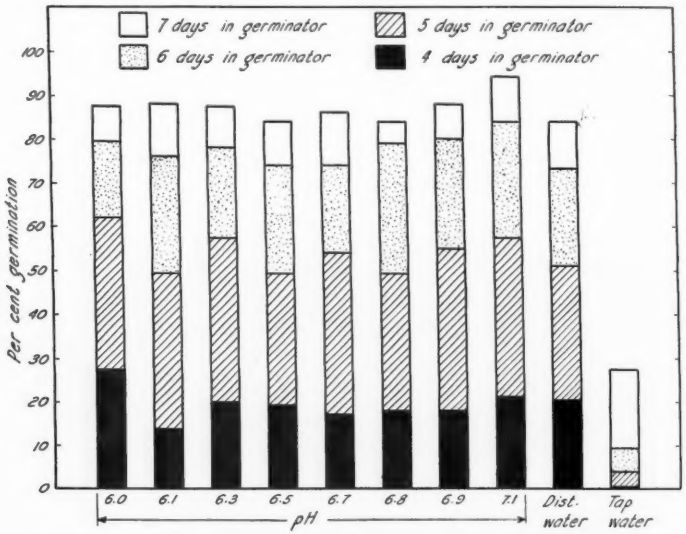

FIG. 5.—Germination in tap water, distilled water, and mixtures of primary and secondary sodium phosphates having a pH range from 6.0 to 7.1. Total concentration of salts in each phosphate buffer solution was $\frac{M}{300}$.

are practically parallel, a slight amount of calcium chloride added to the buffers reduces the germination to that of tap water. Measurements of the pH of buffers with and without calcium chloride showed no significant difference. The reduction in germination must therefore be attributed to the presence of calcium and not to a high pH. Although the tap water (pH 8.4) was much more alkaline than any of the buffers containing calcium (pH range 6 to 7.1), the germinations were almost identical.

WHY DOES CALCIUM RETARD GERMINATION?—To test the possibility that calcium in tap water may in some way modify the per-

meability of the seed coat or other cell layers to water, portions of the seed coat were dissected away to expose a part of the embryo. Although the minuteness of the seeds made dissection without injury impossible in many cases, many normal seedlings were produced in both tap water and distilled, and no significant difference in their

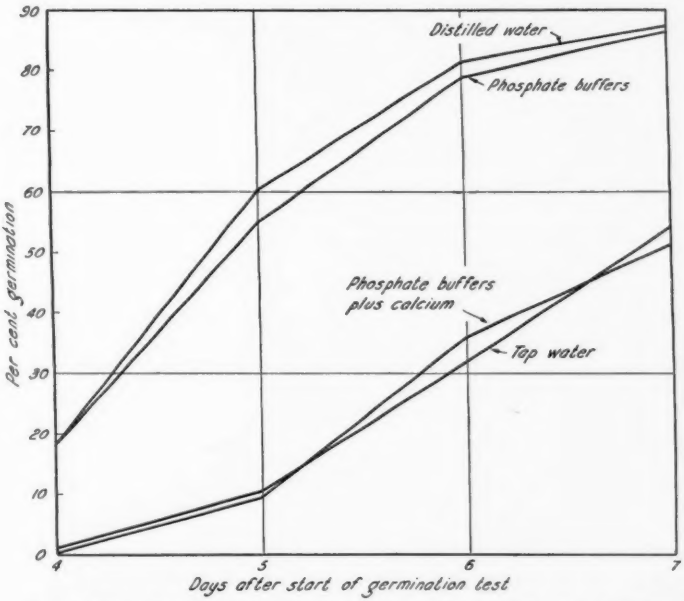

FIG. 6.—Rate of germination in phosphate buffers with and without calcium chloride compared with rate in distilled and tap water, respectively. Buffer curves based on mean germination in eight solutions having a pH range from 6.0 to 7.1. Calcium chloride series contained an amount of calcium approximately double that in tap water.

rates of emergence and growth could be detected. Evidently, then, the tap water does not inhibit germination if it has free access to the embryos; apparently its effect is upon seed-coat permeability.

The rate of water absorption from tap and distilled water respectively was next studied. Although the small size of the seed and the consequent large surface per unit weight make such determinations inaccurate, a significantly greater rate of absorption was nevertheless detected from distilled than from tap water. Two different methods were employed to remove the surface water from the seeds, and

by each of these a more rapid absorption from distilled water was demonstrated.

This difference in rate persists for a very short time. At the end of twelve hours the same amount of absorption occurs in both cases, and during the next few days no further intake is detectable by the methods employed. The short duration of a difference in rate of absorption made it improbable that this factor alone could account for the different rates of germination observed.

Lots of seed were placed in tap water the first day of the test and then transferred to distilled for the remaining six days. Other lots were placed in distilled water the first day, tap water the second day,

TABLE IV

EFFECT ON GERMINATION OF VARIOUS ALTERNATIONS OF TAP AND DISTILLED WATER

TREATMENT	PERCENTAGE GERMINATION			
	4 DAYS	5 DAYS	6 DAYS	7 DAYS
Tap water continuously	1	6	35	56
Distilled water continuously.....	25	60	74	81
Distilled water on all but 1st day.	3	33	60	74
Distilled water on all but 2nd day	5	24	49	70
Distilled water on all but 3rd day.	4	24	56	72

and distilled water again the remaining five days. Still others were kept in distilled water two days, then in tap water the third day, and distilled water again the last four days. Checks were run both in tap and in distilled water.

As table IV shows, all lots placed in tap water one day, whether on the first, second, or third day of the test, germinated at about the same rate; and this rate was intermediate between that of the two checks. Obviously the observed difference in rate of absorption during the first twelve hours is not responsible for the variability in germination rates.

This experiment, however, does not disprove the proposal that tap water alters the permeability of the coats to water, thus retarding germination. The structure of the seed coat indicates that much of the water absorbed during the first twelve hours of soaking is held in its outer layers. If that be true, the amount of water that actually

penetrates the seed coat to the embryo is small, and demonstration of differences in this rate in tap and distilled water would be difficult. Since there is a measurable difference in the initial rate of absorption by the whole seed, one might suspect a difference, difficult or impossible to detect, in the rate of movement of water from the seed coats into the embryo. The role of calcium in delaying germination, however, cannot be conclusively explained from the data presented.

Discussion

The effect of calcium in retarding germination appears rather unusual; the writer is not aware of any other case where such small amounts of calcium have been shown to retard germination. In this connection several other kinds of seed were investigated and were found to be uninfluenced in their rate of germination by the presence of calcium.

TRUE (7) mentions that when a calcium salt is added to distilled water in concentration sufficient to make it osmotically equal to Potomac River water, the cells are in some way protected from the toxic effects of the distilled water. He found that sodium salts, on the other hand, fail to give this protection.

RUDOLFS (5) cites the work of CLAUDEL and CROCHETELLE (1), who found that alkaline substances with Ca or K as a base favor germination. He also mentions that MAQUENNE and DEMOUSSY (4) found calcium sulphate decidedly stimulating in the germination and growth of peas. RUDOLFS found that calcium nitrate affected adversely the germination and root length of nearly all seeds except corn. He presents germination data for only three kinds of seeds treated with calcium, however; and of these only one, white lupine, appears to show any detrimental effect ascribable directly to calcium. Furthermore, it is not conclusive that the difference of 16 per cent shown between the water check and the highest germination obtained after calcium nitrate treatment is significant, because only 50 seeds were used in each test, and the tests apparently were not duplicated.

Small concentrations of calcium may appreciably influence the rate of germination of *Hypericum* and possibly other seeds. Variations in rates of germination of *Hypericum* seeds in different soils and soil extracts do not appear to be correlated with the pH of the soil,

but may be associated with the concentration of calcium. In studying the effects of various toxic agents on germination, one should be aware of this effect and should not confuse it with the effects of the toxic substance.

Summary

1. Germination studies with *Hypericum perforatum* seed showed that tap water retards germination as contrasted with the germination rate in distilled water. Mixtures of the two containing as little as 10 per cent tap water cause as pronounced germination delay as pure tap water. A relatively short period in tap water, followed by transfer to distilled water, also results in a definite retardation.

2. High alkalinity of the tap water and its ionic constitution are both possible causes of the effect observed. The data show that it is not a question of alkalinity, for similar results can be produced in solutions at or below the neutral point. The effects of the various ions present in tap water indicate that calcium causes the retarded germination.

3. No conclusive explanation is offered as to the method of operation of calcium in delaying germination. The data suggest that it alters the permeability of the coat to water.

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STUDIES ON THE EMBRYOGENY OF THE SOLANACEAE. I[1]

P. N. BHADURI

(WITH SIXTY-ONE FIGURES)

Introduction

SOUÈGES (**9**) has studied the embryogeny in some of the genera of the Solanaceae. Considering the large number of genera belonging to this family, however, the literature on the subject appears to be meager. SOUÈGES holds that the embryogeny in the different members of the family follows the same plan of organization. Observations made by the writer on the embryology of some of the members of this family (**2, 4**) indicated a variation in the development of the embryo in *Physalis minima* L., which did not support SOUÈGES' generalizations. A detailed investigation was therefore undertaken to determine the range of variation in the development of the embryo in the same and different species of the Solanaceae. Such a study may be helpful in discovering the phylogenetic relationship between different members of the Solanaceae and the affinities of this family to others. The present paper is therefore only a part of the contemplated work on this subject.

MATERIAL AND METHODS.—The material was obtained from *Physalis minima* L., *Withania somnifera* Dun., *Nicotiana plumbaginifolia* Viv., and *Petunia nyctaginiflora* Juss., grown in the University experimental garden during the year 1931–32. Fruits of different sizes at different stages of development were dissected from healthy plants during bright days, and were killed and fixed, chiefly in Licent's fluid, between 12:30 and 2 P.M. To facilitate penetration, the air was removed from the fluid and the tissue with an exhaust pump, immediately after fixation. The material was then treated as necessary for the paraffin method and sections were cut 6 to 12 μ thick. Heidenhain's iron-alum haematoxylin with or without a counter stain was employed.

[1] Contribution from the Department of Botany, Calcutta University.

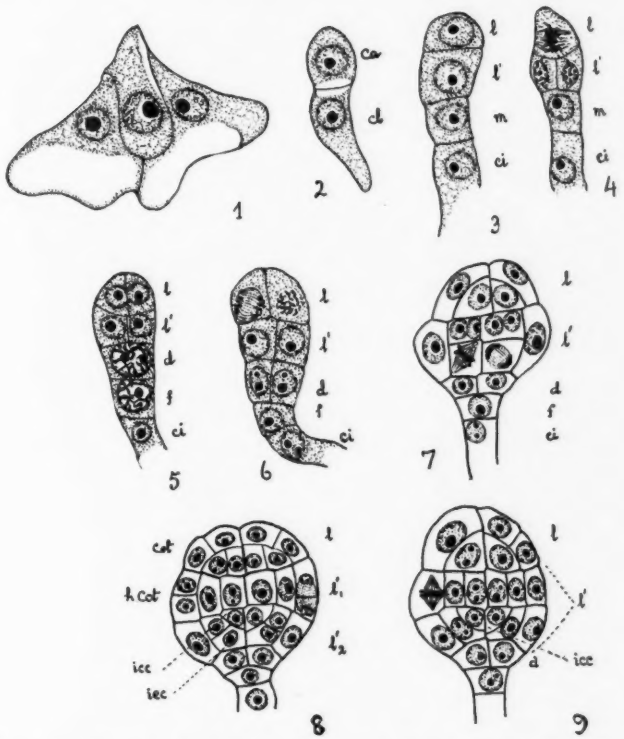
Observations

The time required for the first division of the zygote varies in different genera of the Solanaceae, but the zygote always rests for some time before commencing activity. The endosperm nucleus divides many times, forming a cellular endosperm tissue, before the zygote divides. FERGUSON (6) first showed in *Petunia* that the primary endosperm nucleus divides to form a two-celled endosperm tissue, even prior to discharge of the sperms from the pollen tube. Similar observations have been made by the writer (3) in *Lycopersicum esculentum* Mill. as well as in *Petunia nyctaginiflora* Juss. No trace of synergids has been noted during the first division of the zygote. A quadrant of four endosperm cells is generally cut off by two successive longitudinal divisions of an endosperm cell at right angles to each other; they surround the distal end of the zygote and supply nutrition to it (fig. 1). Development of the embryo begins with elongation of the zygote. The first division is always transverse. The two daughter cells then divide transversely, forming a four-celled linear proembryo (figs. 2, 3, 11, 25–28). The apical cell of the two-celled proembryo may divide either before or after the basal cell divides (figs. 26, 27). According to SOUÈGES (9), the division of the apical cell of the two-celled proembryo generally takes place first. From the present account it will be seen that the basal cell not infrequently divides before the apical cell. In some cases the proembryo begins to bend from the two-celled stage, instead of becoming linear, and looks like an arch in the four-celled stage (fig. 29).

For convenience, the specific cells of the four-celled proembryo are designated as follows: the cell nearest the micropyle is termed *ci;* the next cell above it, *m;* the next cell above this, l'; and the distal cell, l. The first products of l are l_1 and l_2; that of l' are l'_1 and l'_2; that of *m* are *d* and *f;* and the products of *ci* are *n* and n'. These symbols are like those followed by SOUÈGES (9).

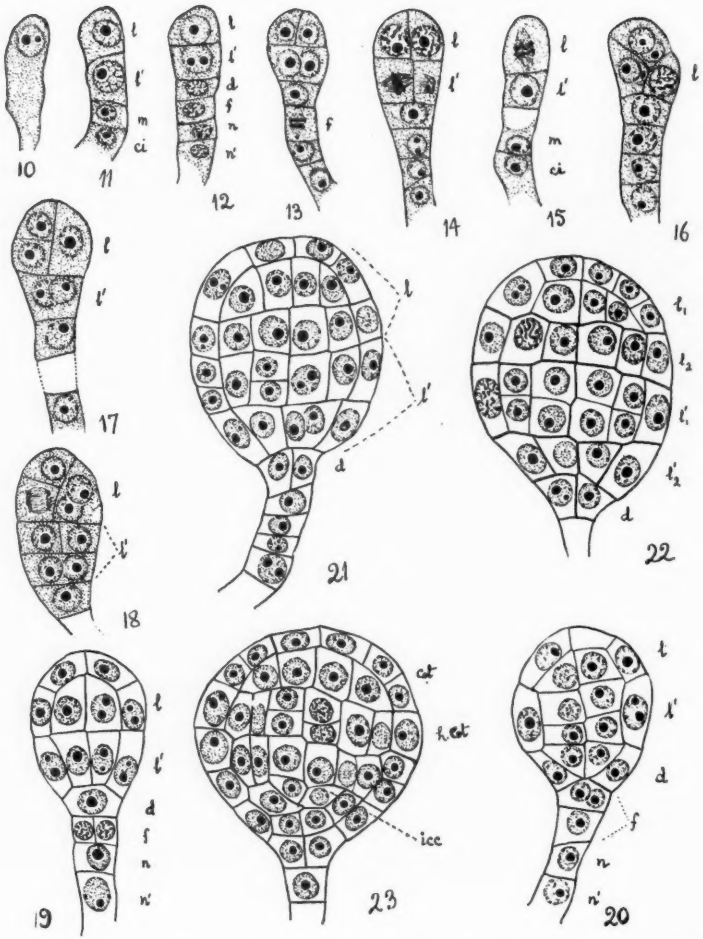
From the four-celled stage, subsequent development generally varies in the different (and sometimes even in the same) species. Four principal types of development have been traced during this investigation, and these four types have all been observed in *Physalis minima.*

The general mode of development of the embryo in *Nicotiana plumbaginifolia*, *Petunia nyctaginiflora*, as well as in some embryos of *Withania somnifera* and *Physalis minima*, corresponds to the



FIGS. 1–9.—Stages in development of embryo in *Petunia nyctaginiflora*. Figure 1 is a longitudinal section through micropylar end of embryo sac, showing two out of the four endosperm cells covering the zygote; they look like synergids. × 600. (*icc*, initials of central cylinder; *iec*, initials of root tip: *cot*, cotyledons; *h.cot*, hypocotyl; explanation of other symbols is given in the text).

"*Nicotiana* type" of development already described (9). In these species the apical cells *l* and *l'* first divide longitudinally, whereas the divisions of *m* and *ci* are transverse. An eight-celled proembryo six cells long is thus produced (figs. 13, 32). Sometimes the longitu-

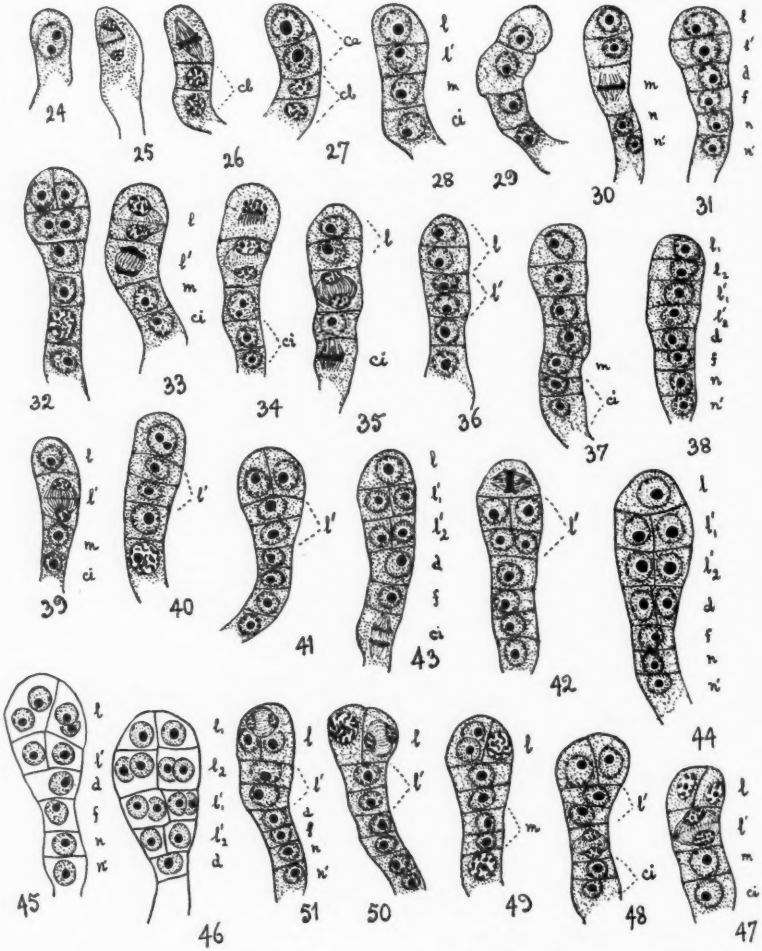


FIGS. 10–23.—Stages in development of embryo in *Withania somnifera:* Figs. 11–13, development of proembryo to six and eight-celled stage; fig. 14, formation of octant stage; figs. 15–18, aberrant type of development of proembryo; fig. 20, differentiation of initials of hypocotyl and root tip from inner cells of *l'*. ×600.

dinal divisions in *l* and *l'* take place before the cells *m* and *ci* divide transversely, whereby a six or seven-celled proembryo four or five cells long is produced (fig. 5). It has been found, as previously noted by SOUÈGES, that the cell *l'* generally divides earlier than the cell *l* (fig. 4). Following the first division of *l* and *l'* they divide longitudinally, forming two tiers of four cells each (figs. 6, 14, 45). The next division in each of these resulting eight cells is periclinal, forming an outer and an inner cell (fig. 19). The outer cells form the dermatogen. These last periclinal divisions have sometimes been observed to take place earlier in the division of *l'* than of *l*. The four inner cells of *l'* then divide transversely, separating the initials of the hypocotyl above and of the radicle below (figs. 20, 53, 56).

In the second type of development, all the four cells *l*, *l'*, *m*, and *ci* divide transversely before any longitudinal walls appear in *l* and *l'* (figs. 33–38). This type of eight-celled proembryo is commonly met with in *Physalis minima*. SOUÈGES (**9**) occasionally found similar proembryos in *Solanum sisymbrifolium*. Further transverse divisions of l_1 and l_2 or l'_1 and l'_2 have not, however, been observed during the present study. The first three or four cells farthest from the micropylar end of the proembryo then divide longitudinally, forming an eleven or twelve-celled proembryo eight cells long. The products of these apical cells then divide again longitudinally. As a result three tiers of four cells each are formed at the distal end of the proembryo (figs. 46, 52). The next division in each of these cells is periclinal, forming an outer and an inner cell (fig. 55), the outer one forming the dermatogen. The transverse divisions leading to the separation of hypocotyl from radicle do not necessarily take place in this type of development.

In the third type of development, the cells *l'*, *m*, and *ci* divide transversely but the first division of the cell *l* is longitudinal (figs. 39–41). The longitudinal division of *l*, however, generally takes place after the transverse divisions in *l'*, *m*, and *ci* are completed, forming an eight-celled proembryo seven cells long. Here also the three distal cells divide longitudinally, twice in succession, forming three tiers of four cells each. Sometimes the longitudinal division in *d* is delayed. Subsequent divisions follow the same sequence as in type two (figs. 42–44, 54).

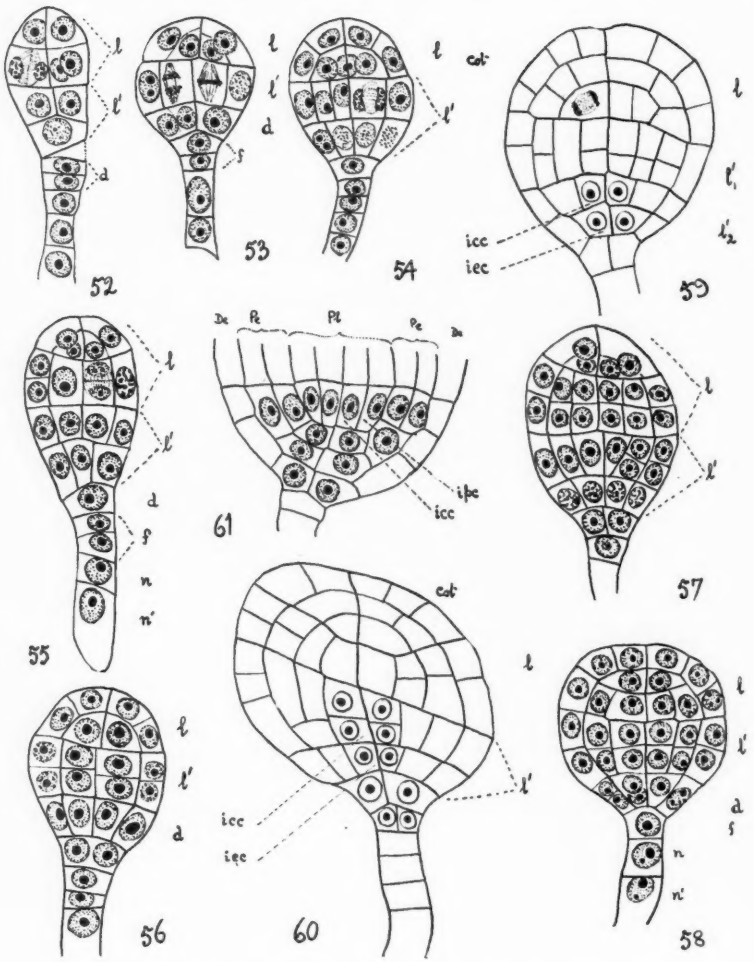


FIGS. 24–51.—Early stages in development of embryo in *Physalis minima:* Figs. 24–29, development of four-celled proembryo from zygote; figs. 30–32, development of six and eight-celled proembryo according to first type of development; figs. 33–38, development of eight-celled linear proembryo according to second type of development; figs. 39–41, development of proembryo according to third type of development; figs. 47–51, development of proembryo following fourth type of development; figs. 43–46, later stages of proembryo according to different types of development. ×600.

In the fourth type of development, the distal cell *l* divides longitudinally in the four-celled stage (fig. 47). The cell *l'*, and also *m* and *ci*, later divide transversely as in the third type of development, forming an eight-celled proembryo seven cells long (fig. 48). The difference in the subsequent divisions of these proembryonal cells from the previous type is that, before any longitudinal division takes place in l'_1 and l'_2, the first products of *l* again divide longitudinally and each of the four cells thus formed divides transversely, forming two tiers of four cells each. The last transverse division is assumed, however, for as soon as this division is completed it becomes very difficult to say whether the apical cells have divided longitudinally twice in succession or a single apical cell has first divided longitudinally twice in succession followed by a transverse division. A critical examination of the sequence of divisions of the individual cells from the four-celled stage, however, leaves no doubt that some of the embryos follow this latter type of development (figs. 49–51).

The octant stage here is thus produced from the activity of a single apical cell, *l*. BORTHWICK (**5**) has described similar aberrant types of embryo development in *Daucus*. Similar divisional stages, as have been shown in the drawings of BORTHWICK, have, however, not been observed. By this time the cell *l'* divides longitudinally and later by another longitudinal division forms a plate of four cells, completing the dermatogen at the radicular apex of the embryo, following a periclinal division.

Differentiation of the initials of the central cylinder from the root cortex, that is, differentiation of the periblem and the plerome at the radicular apex of the embryo, takes place differently in some of the different genera studied. In *Petunia* the origin of the initials of the central cylinder is the result of an oblique division of the inner cell l'_2 (figs. 7, 9). In *Nicotiana*, *Physalis*, and *Withania* the initials of the central cylinder are differentiated by transverse or longitudinal divisions of l'_1 or l'_2 (figs. 23, 57–60). In some embryos differentiation of the initials of the pericycle takes place earlier, owing to longitudinal divisions of the initials of the central cylinder (fig. 61). In exceptional cases differentiation of the initials of the central cylinder



FIGS. 52–61.—Later stages in development of embryo in *Physalis minima* (*ipc*, initials of pericycle; *de*, dermatogen; *pe*, periblem; *pl*, plerome).

has been found to be due to slightly oblique divisions of the products of l'. In the fourth type of development of the embryo, already described, the initials of the central cylinder can originate from products of either l_2 or l'_1.

The relationship of the different organs of the embryo to that of the four cells in the four-celled stage, however, is very different in the four types of embryo development. SOUÈGES (**7, 8**) considers

TABLE I

ORIGIN OF PARTS OF MATURE EMBRYO FROM CELLS OF FOUR-CELLED PROEMBRYO IN THE FOUR TYPES OF EMBRYO DEVELOPMENT

Individual cells of four-celled proembryo	Parts of mature embryo derived from each cell of four-celled stage			
	Type I	Type II	Type III	Type IV
l	Cotyledons	Cotyledons and hypocotyl	Cotyledons	Cotyledons, hypocotyl, and root tip; or cotyledons and hypocotyl
l'	Hypocotyl and root tip	Root tip, root cap, and upper part of suspensor	Hypocotyl and root tip	Root cap and upper part of suspensor
m	Root cap and upper part of suspensor	Suspensor	Root cap and upper part of suspensor	Suspensor
ci	Suspensor	Suspensor	Suspensor	Suspensor

this to be very important in the study of the embryogeny of flowering plants. The differences are shown in table I.

The suspensor of the mature embryo in all the species studied consists of a variable number of cells ranging from five to ten. In *Cestrum diurnum* L., observations regarding the embryogeny of which have not yet been completed, the suspensor is massive and is unlike that found in any other species of the Solanaceae.

The phenomena of polyembryony, under natural conditions, have already been reported by BANERJI and BHADURI (1) in a number of species of the Solanaceae.

Discussion

From a comparative study of the embryogeny of different plants, SOUÈGES (7–11) has concluded that the arrangement of the cells in the four-celled stage and the subsequent behavior of these four cells in the formation of the different parts of the embryo are important essentials in the study of the embryogeny of angiosperms. He has two main types of arrangement of the four cells in the four-celled stage. In the first type two distal cells *l* and *l'* lie side by side and the proembryo is three cells long. This type, commonly found in flowering plants, is a constant feature of the Liliaceae, Ranunculaceae, Cruciferae, Compositae, etc. In the second type a linear proembryo four cells long is formed. This type is common in the Solanaceae, Umbelliferae, Rubiaceae, Scrophulariaceae, etc. From the subsequent behavior of these four cells SOUÈGES has further concluded that each cell of the four-celled proembryo gives rise to a definite region of the mature embryo. This character is mostly common for the different members of the same family or group of plants. It differs, however, in different families and groups of plants. He also holds that the way in which the different parts of the mature embryo are formed from the individual cell of the four-celled stage is very significant in indicating plant relationships.

The study of the embryogeny in the Solanaceae was first made by TOGNINI (12), who described only a few stages in the development of the embryo in *Solanum tuberosum*, *Datura stramonium*, *Physalis edulis*, and *Atropa belladonna*. SOUÈGES (9) studied a number of species belonging to this family and concluded that his generalizations hold good for the entire family. He observed that a linear proembryo is always formed. The present investigation supports this generalization. From the present study, however, it will be seen that the other conclusion of SOUÈGES (9), that definite parts of the mature embryo are always formed from a particular cell of the four-celled proembryo, is not tenable. According to his generalizations, the cotyledons are derived from cell *l*, the hypocotyl and the

root tip from cell *l'*, the root cap and upper part of the suspensor from cell *m*, and the remaining part of the suspensor from cell *ci*. Table I, however, shows that this generalization is incorrect; at least the writer found it so in the case of *Physalis minima*, in which the same part of the embryo originated from different cells of the four-celled proembryo. From the drawings of SOUÈGES (9) it appears that he also observed some stages in *Solanum sisymbrifolium* similar to those seen during the present study. Possibly SOUÈGES has misinterpreted some of the advanced stages of embryo development. In the Umbelliferae SOUÈGES (10, 11) also found a variation in the development of the proembryo in *Carum carvi*, similar to that observed in *Physalis minima*. He concluded, however, that the organs of the mature embryo in these cases also develop from a particular cell of the four-celled proembryo. BORTHWICK (5), on the contrary, has made it clear that this generalization of SOUÈGES regarding the embryogeny of the Umbelliferae also fails in the case of *Daucus carota*. BORTHWICK also thinks that SOUÈGES might have misinterpreted some of the advanced stages of embryo development in *Carum*.

It will be seen from the present observation that the method of origin of the different parts of the mature embryo varies in different and sometimes even in the same species of Solanaceae. From the observations of SOUÈGES as well as from the present study, it will be further seen that the method of differentiation of the initials of the central cylinder is generally fixed for such genera as *Nicotiana*, *Hyoscyamus*, *Atropa*, *Petunia*, etc. In *Solanum*, *Withania*, and *Physalis* a variation in the origin of the initials of the central cylinder and the different parts of the mature embryo is very common.

From a comparative study of the embryology and of the morphological resemblances existing in the Umbelliferae and Solanaceae, SOUÈGES (11) has concluded that a close phylogenetic relationship exists between these two families. There exists much controversy as to the phylogenetic position of the Umbelliferae. SOUÈGES concluded that these two families should be put in the same order, Solanales. The observations of BORTHWICK (5) have, however, weakened SOUÈGES' conclusion. From the present study it will

be seen that the embryogeny of *Physalis minima* is very similar to that observed by BORTHWICK in *Daucus carota*, and that the variation in the embryogeny of the two families appears to be the same. But from the present state of our knowledge regarding the embryogeny of these two families it will not be safe to accept SOUÈGES' theory until further data accumulate on their embryology.

Summary

1. In the light of SOUÈGES' theory, the development of the embryo from the zygote has been studied in *Physalis minima*, *Withania somnifera*, *Nicotiana plumbaginifolia*, and *Petunia nyctaginiflora*.

2. The generalization made by SOUÈGES, that the four cells of the four-celled proembryo always give rise to the same part of the mature embryo, seems to be untenable in the light of this investigation.

3. The variation in the development of the embryo in the Solanaceae and Umbelliferae appears to be the same.

The writer desires to express his thanks to Mr. I. BANERJI for his helpful suggestions and constant encouragement throughout this investigation.

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STUDIES ON AMBROSIA. II: EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON FLORAL DEVELOPMENT OF AMBROSIA ELATIOR

KENNETH L. JONES

(WITH SIXTEEN FIGURES)

This paper is a report on some observations and minor experiments made during the past six years in the course of genetical studies on the floral types of the ragweed, *Ambrosia elatior* L., recently published (4). Plants of this species may be conveniently placed into four categories or forms, based on breeding behavior, and these are briefly listed because the environmental effect depends upon the form used.

MONOECIOUS FORMS

FORM 1.—This is the well known form, widely distributed in nature, in which the racemes are staminate (fig. 1) and the pistillate heads occur in leaf axils. It breeds true, when used as the female parent, regardless of the form of the male parent, which does not affect the floral type in this species.

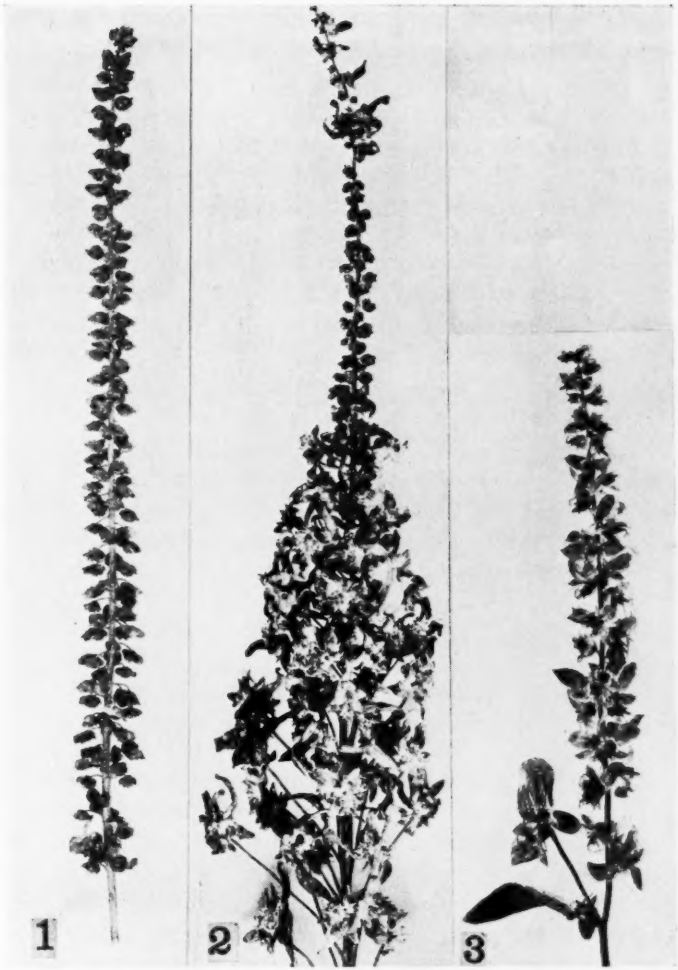
FORM 2.—Identical in appearance to form 1, but does not breed true.

FORM 3.—Racemes contain both staminate and pistillate flowers (fig. 2); does not breed true, but produces more pistillate offspring than does form 2.

PISTILLATE FORM

Racemes pistillate (fig. 3); does not breed true, but produces more pistillate offspring than do the monoecious forms.

It is to be noted that only monoecious form 1 breeds true, and that there are no secondary characteristics to distinguish the forms prior to anthesis, so that it is usually impossible to determine environmental effects on floral development of individual plants. The method employed was to compare ratios of forms in treated cultures with a control grown from seed derived from the same plant.



FIGS. 1–3.—Fig. 1, staminate raceme of monoecious form 1 or 2; fig. 2, androgynous raceme of monoecious form 3; fig. 3, pistillate raceme of pistillate form.

Results

The effects of waterlogged soil, photoperiods of 20 and 24 hours, and the removal of plumules were studied. The results are recorded in table I. The data indicate that the role of the environment on the floral development depends upon the form used, since the offspring of forms 1 and 3 were not altered by any of the treatments, whereas form 2 and P (pistillate) were affected.

TABLE I

Form	Treatment	No. seedlings	No. plants to flower	Percentage		
				1–2*	3	P
1	Control	100	100	100	0	0
1	Waterlogged	100	98	100	0	0
1	20 hour photoperiod	98	40	100	0	0
1	24 hour photoperiod	100	60	100	0	0
1	Plumules removed	60	47	100	0	0
2	Control	209	174	67.8	24.1	8.0
2	Plumules removed	99	90	53.3	32.2	14.4
3	Control	163	145	27.5	34.4	37.9
3	Waterlogged	100	92	28.2	33.6	38.0
P	Control	201	170	1.7	40.5	57.6
P	Waterlogged	100	93	8.6	13.9	77.4
P	20 hour photoperiod	93	22	13.6	4.5	81.8
P	24 hour photoperiod	100	58	5.1	25.8	68.9

* Monoecious forms 1 and 2 are identical in appearance.

The instances of altered ratios require comment. Mutilation, pruning, and the removal of floral buds have often been reported to produce changes in floral development, for example Higgins (3), Murneek (5), and Pritchard (6). The results secured from pruning mature plants of *A. elatior* were unsatisfactory; form 3 alone was altered, and the results depended upon the distribution of the different heads in the racemes. The removal of plumules from seedlings of plants derived from form 2 increased the percentage of pistillate plants. This treatment was not a means of introducing a single variable, of course, for it entirely changed the plants to a dichotomous habit, and arrested their development so that anthesis occurred 2–3 weeks later than the controls which consequently grew under different conditions of temperature, light, and humidity. It is significant

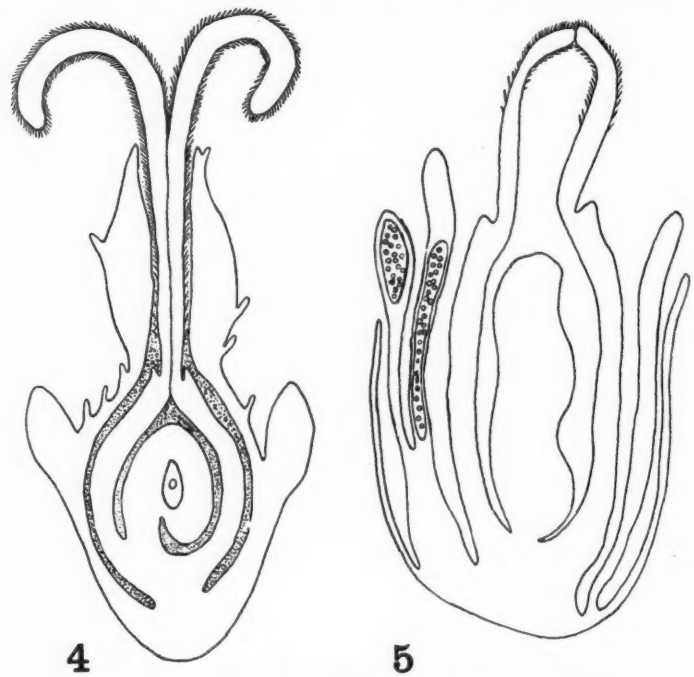
that, in spite of the altered ratios, plants of forms 1–2 predominated and pistillate plants were least abundant in both the control and the culture in which the plumules were removed.

The offspring from a pistillate plant gave very dissimilar ratios when subjected to various treatments, although there was never a differential swing toward the staminate or pistillate condition, since an increase occurred both in the predominantly staminate forms 1–2 and in the pistillate form. The writer had no intention of investigating the effect of waterlogged soil on floral development, but secured these data in testing the effect of a high concentration of Ca^{++}, K^{+}, $PO_4^{\equiv}$, and NO_3^{-} ions. The control culture, kept drenched with rainwater, gave practically the same deviation from the control in the field as those in which the soil was kept saturated with the foregoing nutrients, so that the effect was apparently the result of anaerobic conditions in the waterlogged soil.

The photoperiod is a significant factor in the growth of *A. elatior;* for example, plants (from any form) which flower during the short days of December or January are very dwarfed, and usually fail to produce racemes. They may form a few staminate heads at the terminal growing point, but often produce only lateral flowers, which are always pistillate. Such pistillate plants, in a culture which should be monoecious, cannot be regarded as "sex reversals" since pistillate flowers have not replaced staminate flowers. GARNER and ALLARD (2) grew six plants of *A. elatior* under a photoperiod of seven hours from June 3 to July 1, when pollination began. The plants were returned to normal light conditions and "blossomed a second time during the last week in August, which is also the time of blossoming of the original controls and of ragweed growing in the field. It may be noted, however, that while the original growth produced staminate spikes as well as pistillate flowers in the usual manner, the second growth produced pistillate flowers almost exclusively." Long photoperiods delay flowering; for example, at 20 hours pollination began November 10, whereas the control pollinated August 15. It is possible, of course, to assume that the ratios shifted because form 3 constituted the major portion of the large group of plants which continued to vegetate and died without flowering.

It has been found that racemes, even of form 1, may be modified

if the photoperiod is increased after they have begun to form. Figure 16 shows an extreme case in which a raceme from a form 1 plant developed in turn two pistillate heads, several staminate heads, several pistillate heads some of which were subtended by pinnatifid leaves, and finally staminate heads. This is the only instance in this species

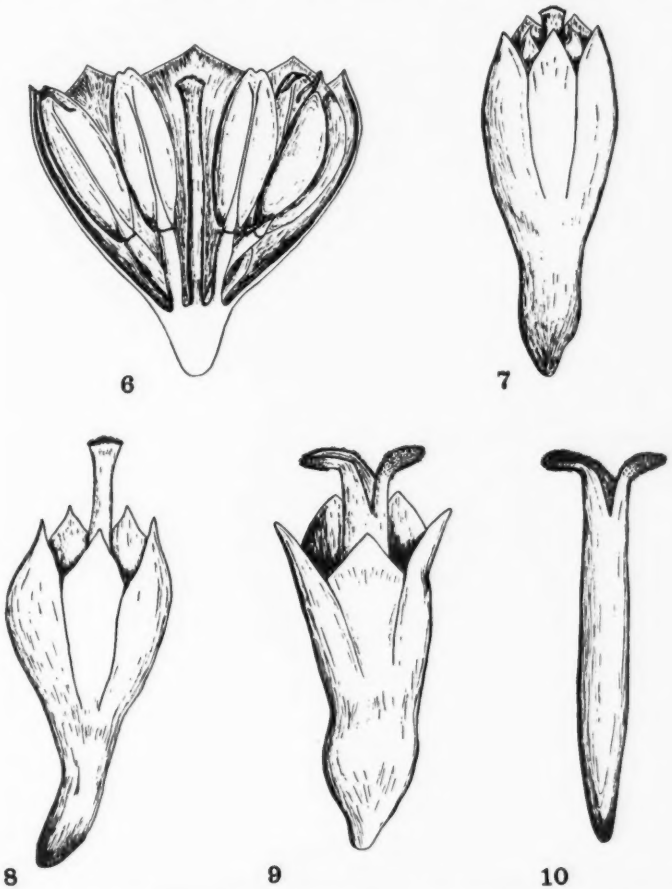


FIGS. 4, 5.—Fig. 4, pistillate head in longitudinal section; fig. 5, diseased pistillate head with two anthers in supernumerary bracts.

in which typical leaves have been observed in an inflorescence, and it is also the sole example of a form 1 plant producing several pistillate heads terminally. Over 10,000 plants of this form have been cultured.

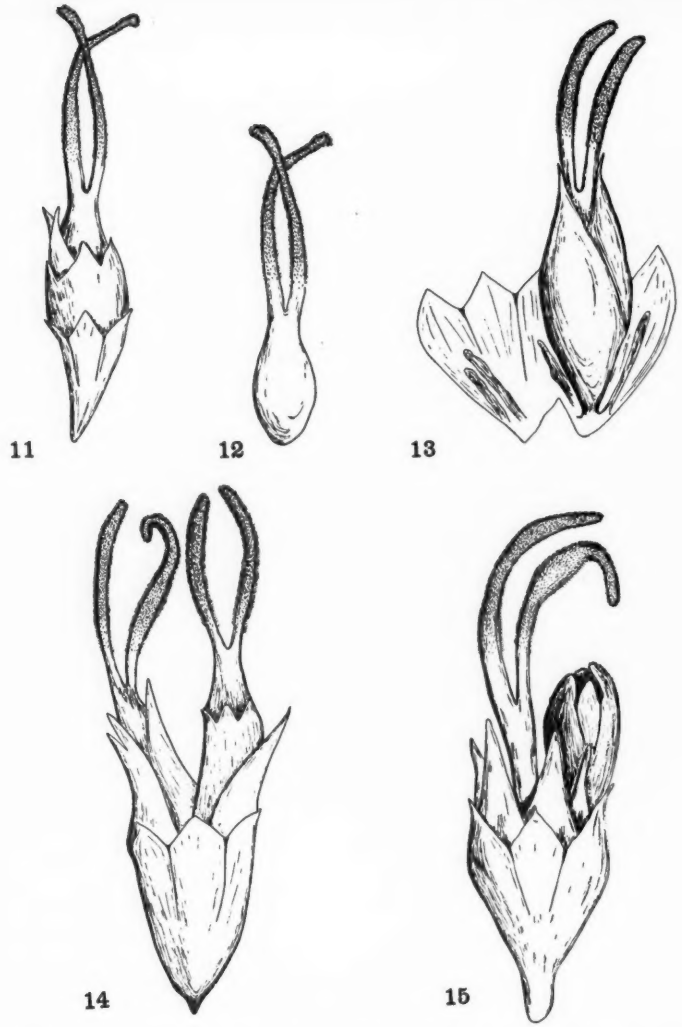
Late in the season, after seeds have set, a few plants of forms 1 and 2 have developed sterile pistillate heads from vestigial pistils of old staminate flowers. A staminate flower (fig. 6) has a perianth of one whorl which is funnelform and five-toothed; the five anthers are

distinct, and surround a vestigial pistil which is uniformly cylindrical except for a dilated tip. As the flower ages, the vestigial pistil



FIGS. 6–10.—Fig. 6, staminate flower in section; five stamens and vestigial pistil; fig. 7, staminate flower, pistillodium elongated; fig. 8, pistillodium further elongated; fig. 9, pistillodium bifurcated; fig. 10, pistillodium of fig. 9.

usually elongates so as to extend beyond the other floral parts (figs. 7, 8). In a few plants the dilated tip bifurcates and continues to elongate into a forked style with stigmatic surfaces, and the base of



FIGS. 11–15.—Fig. 11, pistillate head within staminate flower; two series of bracts, perianth of old staminate flower at base; fig. 12, pistil from fig. 11; fig. 13, staminate flower with perianth dissected to show four old stamens and pistillate head adhering to corolla; fig. 14, staminate flower in which two pistillate heads have formed; fig. 15, staminate flower in which pistillate head and staminate flower have formed. Edges of perianth of new staminate flower have stigmatic surfaces.

the pistillodium enlarges to resemble an ovary (figs. 9–12). As the pistillodium expands, it becomes ensheathed by an involucre of more or less separate bracts which develop adventitiously from the perianth at its base (figs. 11, 13). The pistils have not been found to

FIG. 16.—Androgynous raceme with pinnatifid leaves developed on plant of form 1 by increasing photoperiod after raceme bud appeared.

produce ovules; instead the ovary may be hollow, a solid parenchymatous structure, or it may even harbor an aborted staminate flower. Many variations occur. Figure 14, for example, shows two pistillate heads within the same staminate flower, and figure 15 shows a pistillate head and a staminate flower within the same original staminate flower. In the latter example the newly formed staminate flower possessed stigmatic surfaces along the edges of the corolla.

Several plants of forms 1 and 2 pollinated in May, because of an early planting, and these were continued in culture both out-of-doors and in the greenhouse in order to duplicate the late-season effect. Some plants were heavily fertilized, as SCHAFFNER (**9**) has shown that this often results in a reversal from the staminate to the pistillate condition. Although the plants lived through August, no change in floral development occurred, so that apparently only a very few plants, probably of a certain genotype, can be stimulated to develop pistillate heads within old staminate flowers. ROSA (**8**) reported a similar case in spinach in which the late-season conditions affected the floral type of only one strain.

The writer chanced to observe some sections of pistillate heads of form 1 which produced anthers containing normal appearing pollen within supernumerary bracts (figs. 4, 5). The young seed and certain other tissues of the head had degenerated, although no disease was noticed when the material was collected and no organism can be recognized in the tissues. Lateral pistillate heads elsewhere in the species have shown no staminate rudiments, so that it was surprising to find this modification in the most stable form.

Discussion

The environment has a role in the floral development of *Ambrosia elatior* although this varies greatly with the strain. This has been noted frequently for other species; for example, in *Zea mays* (**1**, **7**) and in spinach (**8**). The ratios of the floral types of cultures derived from monoecious form 2 and pistillate plants were readily altered by the environment, but the predominating type maintained its relative position so that only a few plants could have been affected. In these plants the inherited tendency to produce pistillate heads laterally was maintained, but the action of genes for floral expression in the racemes was changed in certain regions, or sometimes in an entire raceme. The individuals so affected must have had a gene complex somewhat different from their more stable relatives subjected to the same environment.

Monoecious form 1, on the other hand, developed true to type under all conditions, except where the photoperiod was increased after floral buds appeared. In this form the gene complex which

affects the development of staminate and pistillate flowers must be so differential that they formed in definite regions and at a particular time in the ontogeny in most environments. The change produced by disease and by a late season occurred in flowers which had formed and functioned normally. A floral part, like any other structure of a monoecious plant, has potentialities in genes for staminate and pistillate expression, even though one of these has been suppressed, because the time of action of genes is itself inherited. Every staminate flower of *Ambrosia elatior* has a vestigial pistil, which suggests its potential dimorphism, and the environment may accentuate this condition by causing the vestigial pistil to differentiate into a sterile pistillate head.

Summary

1. This report is subsidiary to a genetical study on the floral types of *Ambrosia elatior* L., recently published.

2. The effect of the environment was determined by comparing the ratios of floral types in treated cultures with a control derived from seed of the same plant of known breeding behavior.

3. The influence of the environment in altering the ratios varied with the strain employed. The monoecious form which is widespread in nature was very stable, and was affected only by an increase in photoperiod after floral buds had appeared. Other forms had the ratios of their floral types considerably altered by removal of plumules, by growth under a photoperiod of 20 and 24 hours, and by waterlogged soil. However, the type which predominated in the control always remained dominant in treated cultures.

4. Old staminate flowers of a few plants of different forms developed non-functional pistillate heads as a late season effect.

5. Diseased pistillate heads in the leaf axils developed anthers in supernumerary bracts.

The writer is indebted to Professor H. H. Bartlett for many helpful suggestions.

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LEPIDOCARPON SPORANGIA FROM THE UPPER CARBONIFEROUS OF ILLINOIS

FREDDA D. REED

(WITH TEN FIGURES)

Introduction

Reference to *Lepidocarpon* first appeared in 1877 (**9**), under the name of *Cardiocarpon anomalum*. WILLIAMSON applied the term to some isolated, mature, seed-like structures which in tangential section are in such close agreement with *Cardiocarpon* that CARRUTHERS (**9**) believed them to be identical with his *Cardiocarpon anomalum*, a genus of undoubted gymnospermous origin. At the time WILLIAMSON expressed some doubts as to the identity of the two specimens, doubts which were later justified by the investigations of SCOTT. In 1900 WILD and LOMAX (**7**) established the lycopodiaceous affinity of the cone of WILLIAMSON's *Cardiocarpon*. Somewhat later SCOTT (**6**), working with the same and additional material, substantiated the discoveries of WILD and LOMAX, and further described a strobilus exhibiting differential development. This strobilus, bearing mature, integumented *Cardiocarpon*-like megasporangia at the base and immature lepidostroboid megasporangia at the top, confirmed the relationship of the previously described isolated sporangia. The axis of this cone and the sporophylls were not to be distinguished from the axis and sporophylls of *Lepidostrobus*, but SCOTT believed that the fructification as a whole was "too distinct to be simply left in that genus," and so he established the generic term *Lepidocarpon* for its reception.

Isolated specimens of *Lepidocarpon* have been found in American coal ball material. KRICK (**4**) described *L. lomaxi* found in a coal ball from coal no. 5, Harrisburg, Illinois. These specimens were mature sporangia enveloped by the lamina-like processes which have their origin in the pedicel of the sporophyll; it is at this stage in the ontogeny of *Lepidocarpon* that sections, cut tangentially to the axis of the cone, bear a superficial resemblance to longitudinal sections of

integumented ovules of some primitive gymnosperms, for example, *Cardiocarpon*.

Other specimens have been found in coal no. 6, Petersburg, Indiana (5). They were immature sporangia detached from the strobilus before the delayed laminal development, and in so far as could be determined, their general topography and structure more nearly approximated the sporangia and sporophylls of *Lepidostrobus*. The possession of a single functioning megaspore, almost filling the sporangial cavity, established this latter form in the genus *Lepidocarpon*.

Material

The *Lepidocarpon* sporangia here described were found in coal ball no. 236 of the Harrisburg collection. The collection was made by Dr. A. C. Noé under the auspices of the Illinois State Geological Survey, from coal seam no. 5, Harrisburg, Illinois, which belongs in the Upper Pennsylvanian horizon. The sections are now a part of the paleobotanical collection, Department of Botany, University of Chicago.

The coal ball was a heterogeneous mass of plant structures imbedded in a matrix consisting largely of calcium carbonate and pyrite. In it were identified fragments of stems, roots, leaves, and sporangia of different species of ferns; stem tip, root, and leaf of *Sphenophyllum;* leaves and cortical tissue of *Lepidodendron;* petiole of *Medullosa;* and leaves of Cordaites in addition to some sixty specimens of *Lepidocarpon* sporangia. These sporangia were detached from the axis of the strobilus and separated from one another. They varied in size as well as in age; but remembering the acropetal development and the wide variation in the size and in the stage of maturation of the sporangia contained in the strobilus described by SCOTT (6), one can regard these sporangia as having originated from the disintegration of a single cone.

Description

SPOROPHYLL.—The sporophyll is of the usual *Lepidostrobus* type, consisting of a radially extended part (radial with respect to the axis of the strobilus), referred to subsequently as the pedicel, and the upturned leaf-like laminal portion. In its general topography it is con-

formable with that of *L. lomaxi* already mentioned and reproduced in figure 1. Figures 2, 3, and 4, from the Harrisburg material, are diagrams of separate sporangia but represent tangential sections from the proximal, middle, and distal regions of the pedicel or from the levels *a*—*a*, *b*—*b*, and *c*—*c* of figure 1. Figure 2, a somewhat oblique and crushed tangential section, shows the prominent dorsal

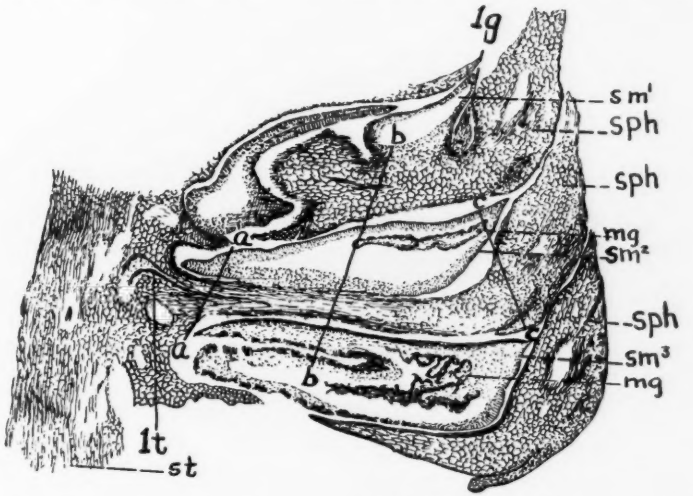
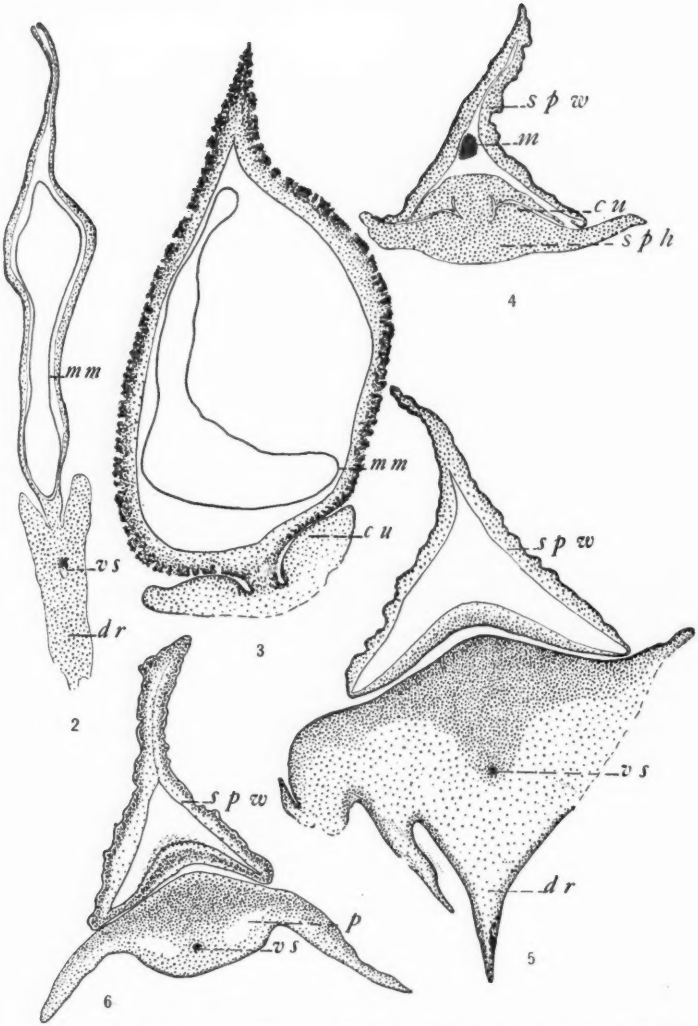


FIG. 1.—*Lepidocarpon lomaxi*, approximately radial section of strobilus: *st*, stele of axis in oblique section; *lt*, leaf trace bundle; *sph*, sporophylls, of which three are shown, each with horizontal pedicel and ascending lamina; *sm*¹, *sm*², *sm*³, sporangia belonging to the three sporophylls; *lg*, ligule; *mg*, remains of megaspore. ×21. After SCOTT (6).

rib; at the proximal end of the pedicel it forms a keel-like projection which becomes confluent with the axis of the cone. For most of its length the pedicel is much thinner vertically, but the margins are expanded into lateral cushions (*cu*, figs. 3, 4) which partially surround and presumably support the base of the sporangium. Toward its distal end the pedicel becomes massive, in transverse section appearing as a broad triangle with lateral wings (fig. 5); at this level the sporophyll not only invests its sporangium but the abaxial surface fits neatly over the sporangium below. From this broad triangular zone the sporophyll bends at right angles, forming the



FIGS. 2–6.—*Lepidocarpon*. Fig. 2, oblique tangential section of sporophyll and sporangium from proximal region: *dr*, dorsal rib; *vs*, vascular strand; *mm*, megaspore membrane. ×14. Fig. 3, tangential section of sporophyll and sporangium through middle region: *cu*, lateral cushions of sporophyll. ×12.5. Figs. 4–6, tangential sections through distal extremities of sporangia and sporophylls: *sph*, sporophyll; *spw*, sporangial wall. ×14.

laminal extension which covers the end of the sporangium and overlaps the sporophylls above. Thus the pattern of the sporophyll is so designed that the assemblage would indicate a relatively tight and compact cone with closely enveloped sporangia.

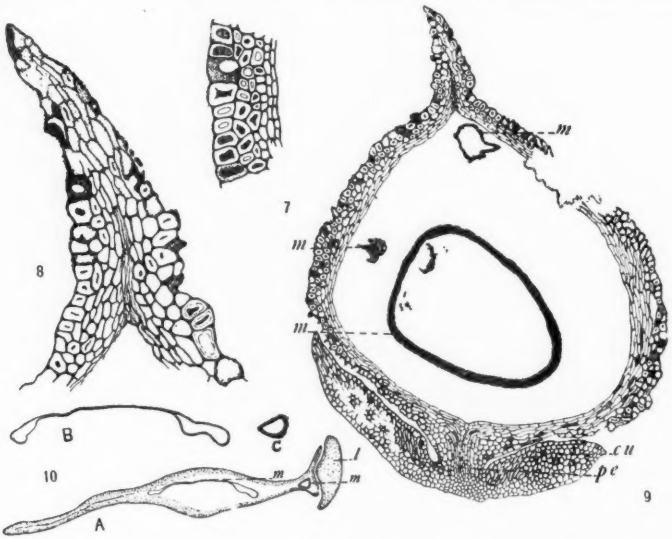
A single vascular strand traverses the length of the sporophyll; in this material it is so poorly preserved, however, that there is no remaining detail of structure, only the course of the strand being indicated. Figure 6, a transverse section of the lamina, shows two lacunar areas occupying the position of parichnos of vegetative leaves of *Lepidodendron;* and since SCOTT, working with more perfectly preserved material, found identical structures in the lamina and vegetative leaves, there seems no reason for doubting the parichnos origin of these areas. In *L. lomaxi* (fig. 1) there is a ligule situated on the adaxial surface of the sporophyll between the sporangium and the lamina. Failure to find any trace of a ligule here is not to be attributed to its absence in the living material, but rather to lack of preservation.

SPORANGIUM.—Located on the adaxial surface of the pedicel and fitting into the groove made by the lateral cushions is the sporangium. Its attachment is a very narrow one tangentially (figs. 3, 4, 9), but radially it extends almost the length of the horizontal portion of the pedicel. The sporangium is elongated radially and deep vertically; it is broad at the base but narrows sharply toward the top, resulting in a sharp ridge which gives the tangential section the appearance of a sporangium with a pointed apex. At the proximal end the sporangium is round at the base, toward the distal end it follows the curvature made by the marginal wings of the sporophyll, and at this level it is triangular in tangential section with the two basal angles, as well as the apical angle, sharply acute (figs. 4–6).

The sporangial wall, which at the basal end merges imperceptibly with the tissues of the pedicel, is of two layers (figs. 7, 8), each averaging about three cells thick. The epidermis is scarcely to be distinguished from the hypodermis, both being composed of thick walled, roughly isodiametric cells, except perhaps for the slightly thicker walls and the larger size of the cells. It does not have the distinct palisade appearance of *L. lomaxi*, or as described for *Lepidostrobus* (2). The cells of the inner layer are thin walled and elongated

in the plane of the surface of the sporangium (fig. 9). There is no trace of a tapetal layer, nor is there any indication of a sub-archesporial pad; on the contrary the more nearly median sections show a slight furrowing at the base of the sporangial cavity (figs. 3, 9).

MEGASPORE.—In the younger sporangia vestiges of all four spores are found, three of which are shown in figure 9, and their position



FIGS. 7–10.—*Lepidocarpon*. Figs. 7, 8, detail of portion of sporangial wall. ×43. Fig. 9, tangential section of sporophyll and sporangium showing functioning megaspore and two of three aborting ones: *pe*, pedicel of sporangium, semidiagrammatic. ×20. Fig. 10 (*A*), section of sporophyll and sporangium transverse to axis of parent cone: *l*, lamina. ×14. (*B*, *C*), functioning and aborting megaspores from *A* showing difference in thickness of megaspore membranes. ×55.

with relation to one another suggests their origin from the tetrahedral type of division of the megaspore mother cell as usually occurs in pteridophytes. The size of the three aborting megaspores and the thickness of their membranes show clearly that they did not disintegrate immediately after inception, but that there was a period of growth and development of all four megaspores alike during their early history. Soon, however, the proximal spore gained ascendancy

over the other three (fig. 9) and increased enormously in size, at length filling the sporangial cavity (fig. 3). Meanwhile the sporangium had increased in size so that the largest of the spores (fig. 3) are more than twice the diameter of the one shown in figure 9. With increase in diameter of the functioning spores there is a corresponding decrease in the thickness of the megaspore membrane; that of the megaspore of figure 9 is at least 50 μ thick, whereas the membrane shown in figure 10 measures about 18 μ. Again, figure 10 shows the variation in thickness of the membranes of the functional megaspore and one of the aborting megaspores of the same sporangium. In the largest of the sporangia the megaspore membrane is usually collapsed (fig. 3), or it may appear broken and fragmented, again it may have disappeared altogether; only rarely is it ever seen intact and certainly never did I find any tissue that could be interpreted as gametophyte. There are fragments of tissue occasionally met with inside the membrane, but it is clearly intruded material.

Discussion

It has been stated of *Selaginella* (3), "The retention of the spores within the sporangium during gametophyte development, fertilization, and embryo formation suggests seed structure, especially where but one spore of the tetrad develops." Again (1), "In extreme cases there is little dehiscence of the sporangium and the megaspore remains inside, so that the shoot with its cotyledons and stem tip, and the root, break through the sporangium. In this extreme case the term "seed" is strictly applicable to *Selaginella*." True, in such cases, the megaspore has been retained and under such conditions the term seed might seem to be applicable. But in *Selaginella* the sporangial wall is so constituted that it could be of little or no further value in the nutrition of the gametophyte. The developmental story of the megaspore membrane is one of increasing complexity and thickness, with consequent increase in the difficulty of food absorption from the surrounding tissues. Furthermore, the volume of the sporangium is much greater than that of the functioning spore or spores, so that they are free in a big cavity, which would likewise increase the difficulty of food assimilation from the sporangium even though it were available. Moreover, in instances when only one of

the four megaspores becomes functional, its selection appears to be wholly fortuitous and not determined by any particular advantage of position within the sporangium. Consequently, the megaspores of *Selaginella* are of necessity wholly independent of the parent plant for their further development (that is, formation of the archegonium, fertilization, and evolution of the embryo), and any cases of their retention must be attributed to some external ecological factor which would prevent the dehiscence and collapse of the sporangial wall with the resulting liberation of the spores. Under such circumstances there is no physiological relationship existing between the spore and the sporangium and its retention is only apparent, not real.

On the other hand, while these specimens of *Lepidocarpon* were on the whole not so well preserved as those from the English Coal Measures, yet in the preceding description there are a few salient features of these young "un-integumented" sporangia that demonstrate the advance made by Paleozoic pteridophytes, and indicate the stages, beyond the acquisition of heterospory, through which spermatophytes must have passed in attaining the seed habit. They are: (1) the relatively massive sporangial wall, differentiated into an outer protective layer and an inner nutritive layer; (2) the early selection of the chalazal megaspore for the functioning megaspore; (3) the position of the megaspore membrane in contact with the sporangial wall, making possible the assimilation of food; and (4) the decrease in the thickness of the megaspore membrane, thereby facilitating food assimilation. Thus, although we have as yet no material evidence of the retention of the megaspore of *Lepidocarpon*, yet possession of these characters would result in the production of a dependent gametophyte surrounded by living and functional tissue and physiologically retained within it.

Summary

1. Some young "un-integumented" specimens of *Lepidocarpon* from the Harrisburg, Illinois, coal ball collection are described. The geological horizon of the material is Upper Pennsylvanian.

2. The sporophylls consist of a radially extended stalk which at the proximal end is decurrent on the axis of the strobilus, and dis-

tally broadens into the leaf-like laminal portion. The lamina, extending at right angles to the stalk, covers the end of its sporangium and overlaps the sporophylls above. The sporophyll is traversed by a single vascular strand.

3. The sporangia, borne singly on the adaxial surface of the stalk of the sporophyll, are distinguished by the possession of a massive wall differentiated into two general layers, an outer protective layer and an inner nutritive layer.

4. Evidences were found of four megaspores in a sporangium, three of which aborted. The differential development displayed by the various specimens indicated an early selection of the chalazal megaspore as the functioning megaspore. With increase in size of the functioning megaspore there was a progressive decrease in the thickness of the megaspore membrane.

5. From an analogy of developing sporangia and megaspores of *Selaginella* and *Lepidocarpon*, the following conclusions are derived: any case of a megaspore of *Selaginella* remaining within the sporangium is due to some chance external factor and hence is no nearer the seed habit than the case of a megaspore that is shed; whereas, to *Lepidocarpon*, a sporangium of living and functional tissue containing a dependent gametophyte, the term "seed" seems "strictly applicable."

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DEVELOPMENT OF THE MEGAGAMETOPHYTE AND EMBRYO OF ALLIUM MUTABILE[1]

THOMAS REGINALD PORTER

(WITH TWENTY-SEVEN FIGURES)

Introduction

This work was begun with the idea of comparing the developmental morphology of the wild onions (*Allium canadense* L. and *A. mutabile* Michx.) growing around Lincoln, Nebraska, with that of *Allium cepa* L. described by HOFFMAN (3). The development of the megagametophyte of one of them proved so interesting that the emphasis was placed there. Apparently these species of *Allium* have not been previously investigated.

At first both *Allium canadense* and *A. mutabile* were utilized, but the former was abandoned because it produced bulblets and only a few flowers. The latter always produced seeds in abundance.

Material was collected in 1934 and 1935; that collected in the late spring of 1934 proved unsatisfactory because of continuous drought. The material collected during the season of 1935 was reasonably satisfactory although it endured drought late in the season. The abbreviation of the development of the megagametophyte described later might be due to weather conditions, but it appeared to be the usual one.

During a period extending from May to July in 1935, flower buds in all stages were collected and fixed. Formal-acetic-alcohol of the following formula was employed: neutral formalin, 10 cc.; glacial acetic acid, 5 cc.; 50% alcohol, 100 cc. Because of the drought, care was taken to effect complete hydration of tissues before fixation. All material was imbedded in paraffin with the exception of some seeds and embryos. These were cleared in cedar oil and observed thus.

Young flower buds and ovules were sectioned 8–12 μ thick; more mature ovules and the embryos were cut 3–5 μ thick. The greatest

[1] Contribution no. 100 from the Department of Botany, University of Nebraska.

difficulty in sectioning came when the older ovules were sectioned. This difficulty was overcome somewhat by prolonged periods in xylol and xylol-paraffin solutions.

Investigation

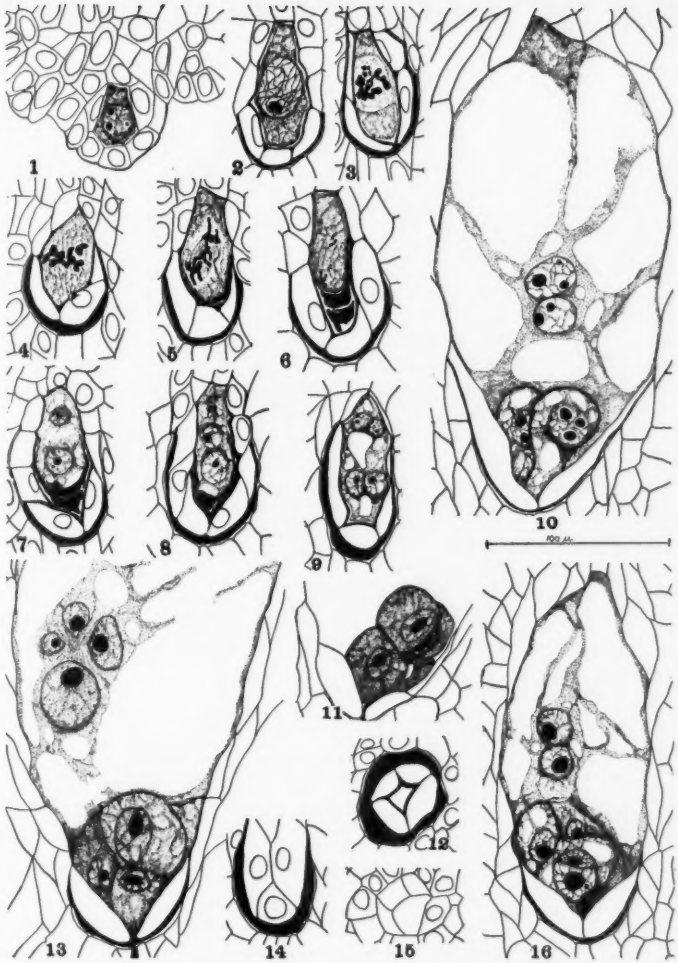
Pollination experiments showed that in all cases seeds matured whether the flowers were cross or self pollinated. The most efficient method of controlling pollination was by tying squares of cellophane over the flower heads, making a pouch with ample space. Superfluous flowers were removed. Material from these experimental heads was fixed every six or twelve hours for a period of six days; then every day for a period of several weeks.

OVULE.—The ovule is essentially of the *Lilium* type. It arises at the base of the ovary, is anatropous, and the funiculus is short. At the time the archesporium becomes evident the rudiments of the inner integument (fig. 1) are fairly well formed and the outer integument is beginning to develop. Both integuments have enveloped the ovule completely before meiosis. The inner integument is from two to four cells thick while the outer attains a thickness of nine cells. At the micropyle the nucellar tissue is separated from the integuments by a distance of about 2–3 μ. At maturity the inner integuments are shorter than the outer. Both are tightly pressed together. At the time of pollination the micropyle dilates. Immediately after the entrance of the pollen tube the integuments again become pressed together.

At maturity, the micropylar and chalazal ends of the megagametophyte are parallel to the funiculus. After fertilization, as the embryo develops, the megagametophyte becomes horseshoe-shaped and widens at the chalazal end to about three times the width of the micropylar end. Later the chalazal end becomes bifurcated by ingrowing sporophytic tissue.

Of the six ovules forming in the young pistil, usually only two or three reach maturity. Ordinarily abortions start about the time that fertilization would normally take place. In some cases as many as six ovules mature.

NUCELLUS.—The nucellus is unusual in that the cells at the extreme micropylar end are larger, thicker walled, and differently



FIGS. 1–16.—Fig. 1, young ovule showing archesporium; figs. 2–4, archesporium undergoing meiosis, outer nucellar walls thickened; figs. 5–7, first division of functional megaspore, one disintegrating; fig. 8, second division in megagametophyte; spindle apparent between small antipodal nuclei; fig. 9, four megagametophyte nuclei before orientation and after disintegration of antipodals; fig. 10, mature megagametophyte; fig. 11, three-celled embryo, pollen tube persisting at right; fig. 12, cross section of nucellar cells at micropyle; fig. 13, two celled embryo, synergid at left, empty pollen tube at right, endosperm cells above; fig. 14, longitudinal view of nucellar cells at micropyle; fig. 15, end view of nucellar cells at micropyle; fig. 16, fertilization, sperm nucleus pressed against egg, synergid on left of egg, pollen tube with second male nucleus on right of egg, disintegrating tube nucleus near micropyle.

shaped from those of the rest of the nucellus. There are only four which differ greatly from the rest. Two of these are long cells which are blunt at one end and taper to the other (figs. 14, 15). The other two are equally large but pointed at both ends, and fit between the two cells having blunt ends (fig. 14). In cross section they are all of similar diameter (fig. 12). These cells are forced apart as the pollen tube enters, and after its protoplasm has moved into the megagametophyte they again come together (fig. 16).

The outer walls of these large nucellar cells are becoming thickened at the time of archesporial differentiation. These cell walls, as well as those of the sporophytic cells in contact with the rest of the megagametophyte (fig. 2), continue to thicken. They reach their greatest thickness at the time the nuclei of the megagametophyte are becoming oriented (fig. 9). The thickening gives the appearance of gelatinous layers. The walls in contact with the megagametophyte remain thickened until reduced by its enlargement as it approaches maturity. This decrease in thickness of the cell walls may be due in part to the absorption of some of the material by the enlarging female nuclei, as suggested by COULTER and CHAMBERLAIN (1). In these illustrations the heavy nucellar walls are shown with a single, heavy black line.

MEGAGAMETOPHYTE.—The archesporial cell is differentiated about three or four days before the flower bud opens (figs. 1, 2). It arises from a hypodermal cell and differs from the rest of the cells in density of protoplasm and in size of cell and nucleus. No case was found in which more than one mother cell occurred. No primary parietal cell is formed, the first division being meiotic (figs. 3–5) and resulting in two cells separated by a wall. The one nearest the micropyle immediately disintegrates; the other undergoes one more division, thus forming two megaspores separated by a wall. The one near the micropylar end disintegrates at once (figs. 6, 7).

The remaining functional megaspore undergoes an unequal division, resulting in a large nucleus at the micropylar end and a smaller one at the chalazal end (fig. 7). After this division the megagametophyte enlarges, thus forcing the disintegrating megaspore toward the micropylar end and leaving it in finger-like strands along the wall of the gametophyte (figs. 6–8).

The division of the two nuclei already formed results in two small, ephemeral nuclei at the chalazal end and two larger, unequal ones at the micropylar end (fig. 8). The larger nucleus of these two is nearer the micropyle. Each of these two functional nuclei divides once more. The innermost produces two daughter nuclei of equal size which function as the two polar nuclei. The micropylar one produces two nuclei, one of which is slightly larger than the other (fig. 9). This larger nucleus becomes the egg; its sister nucleus, the long-lived synergid. By vacuolization of the cytoplasm these four nuclei become oriented as in figure 9. The disintegrating megaspore now becomes absorbed completely. Possibly its substance nourishes the megagametophyte. This stage was found most commonly in material collected a few hours before the flower opened. Between this stage and the one at which the megagametophyte is matured, these four nuclei become very different in appearance. The two polar nuclei are pale bodies with little chromatic material; the egg and synergid are of about the same density but can be distinguished by differences in size and vacuolization. They are separated from the surrounding protoplasm by membranes. The egg cell has a large vacuole between its nucleus and the micropyle, whereas the synergid is vacuolated only at the end farthest from the micropyle. The egg lies nearer the periphery of the megagametophyte than does the synergid (fig. 10).

When the megagametophyte has enlarged about fifteen diameters after its last nuclear division, the gamete is ready for fertilization. Now the megagametophyte is more or less elliptical, but slightly narrower and more pointed at the extreme chalazal end. By growth of the rest of the gametophyte this antipodal region at the chalazal end becomes a small pocket pressed into the surrounding tissue (figs. 10, 16). Because of further vacuolization in the cytoplasm of the gametophyte it enlarges still more. Most of the cytoplasm is massed around the egg and synergid, where it is also the most dense. A smaller amount is gathered about the polar nuclei and a still smaller amount lies at the chalazal end. The masses of cytoplasm are connected with each other and the peripheral cytoplasm by long, irregular strands. The megagametophyte was found to have reached maturity about six hours before the first anther opened, or about six hours after the flower had opened.

It was found from observing many megagametophytes that they range in size from 175 to 300 μ in length, and 78–123 μ in width. The larger gametophytes were found in pistils in which the greatest number of ovules had aborted.

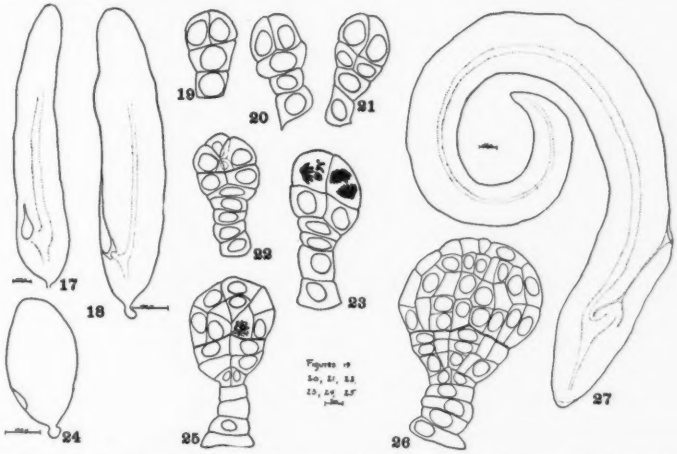
FERTILIZATION.—Fertilization occurs between twelve and twenty-four hours after the flower opens. From the papillate nutritive cells at the base of the ovule the pollen tube passes directly into the micropyle. The tube passes through it, forces apart the large nucellar cells at the micropylar end of the megagametophyte and enters on the side opposite the synergid, against which it presses. The first male nucleus passes into the cytoplasm of the egg and becomes tightly pressed against the egg nucleus (fig. 16). In this figure the second male nucleus may be seen in the pollen tube on its way to fuse with the two polar nuclei. The tube nucleus which is starting to disintegrate may be seen as a black spot between the nucellus and the egg. The pollen tube persists for some time, usually until after the embryo has reached the three celled stage (fig. 11).

At the time fertilization occurs the synergid is rich in protoplasm. It persists in this condition until the embryo has reached the two or three celled stage (fig. 13). This is between forty-eight and seventy-two hours after fertilization.

The polar nuclei become closely applied to each other by the time the second male nucleus has reached them. After triple fusion the endosperm nuclei divide rapidly for a time before the zygote divides. There is an unequal division here which produces endosperm nuclei of slightly different sizes. Figure 13 shows one complete endosperm nucleus and parts of three others. The spindle is still apparent between the new nuclei and they seem to be pulled to a point on the side toward the spindle. These nuclei soon migrate to the periphery of the sac, where they continue to divide. At maturity they are evenly distributed through the sac and about the embryo. Finally they become the nuclei of the usual cellular endosperm.

After fertilization, the outer integuments close together again as do also the nucellar cells at the micropylar end, although the latter are slower in closing. Immediately the megagametophyte begins to elongate at the chalazal end, doubles back toward the micropyle, and becomes horseshoe-shaped.

EMBRYO.—The first division of the zygote takes place about forty-eight hours after fertilization, and is transverse and unequal (fig. 13). The cell toward the micropyle is smaller and often irregular in shape, being pushed in on one side by the pollen tube and on the other by the synergid. The terminal cell is usually about twice as large as its sister cell. The latter will divide to form the suspensor and part of the embryo.



FIGS. 17–27.—Figs. 17, 18, 24, 27, successive stages in development of embryo; figs. 19–23, 25, 26, detail of early stages in development of embryo.

The second division in the terminal cell (fig. 11) is at right angles to the plane of the first division. This occurs sixty to seventy-two hours after fertilization. Usually these cells are oval in shape, with nuclei longer than they are wide.

The second division of the suspensor initial (fig. 19) forms a two-celled suspensor. The next division occurs in the cell adjoining the embryo and contributes one cell to the base of the embryo (fig. 20). This cell continues to divide to form the radicle and its parts. The two terminal cells of the embryo divide to form the cotyledon and stem meristem. The region in which the notch will develop is between the terminal cell and the one cut off from the suspensor (figs. 21, 22, and following). At maturity the suspensor is several cells long and two cells wide and thick (figs. 18, 24).

From this stage on it is hard to determine the sequence of the divisions since they take place rapidly. Figure 23 shows the mitotic division which is the beginning of differentiation of the primary histogens. At the stage shown in figure 25 the place where the notch will form in the embryo is becoming apparent (on the right side). At this stage the dermatogen has become differentiated over the cotyledon. Just above the fourth suspensor cell are the three layers of cells which develop into the radicle and the region producing the stem tip.

Figure 26 shows the enlarged embryo and further differentiation of the tissues. The dermatogen is evident extending around the embryo. Directly beneath the dermatogen is the periblem which is about two cells wide in the upper part and one in the lower. In the central portion of the embryo are the cells which form the plerome. The upper part of the embryo develops with great rapidity to form the long, coiled cotyledon; whereas the lower part, from the notch down, is somewhat slow in growth. Figure 24 shows the embryo advanced to a further stage. This surface view shows the notch well developed. Figure 18 shows the embryo in a still more advanced stage. The notch is nearly complete. The meristematic stem tip is now pushing up into the notch. The dotted lines indicate where the procambial strand has started to develop. This figure, as well as figures 17, 24, and 27, was drawn from entire embryos which had been cleared in cedar oil. Figure 17 shows the embryo further developed. The procambium, which appears as a dark band through the cleared tissue, is extending well toward the cotyledonary tip. The tip of the cotyledon is starting to curve backward. In figure 27 the mature embryo is shown.

The mature embryo lies curled around in the seed. It is completely surrounded by hard, starchy endosperm and in all respects is much like the one described for *Allium cepa* by HOFFMAN (3). The procambium lies in the same general position in the embryo, but that of the plumule is found to extend farther toward its tip than it does in *A. cepa*. It extends to the tip of the cotyledon, suggesting that the cotyledonary tip may be haustorial in nature. In this embryo the stem tip is more closely surrounded by tissue than is the one described by HOFFMAN. Furthermore, the notch is much deeper in this

species and is in the form of a narrow tube, widening at both ends (fig. 27). It does not have the characteristic tip described for the other species. The embryo of this species needs further investigation.

Discussion

A number of variations have been reported in the development of the megaspores of monocotyledons (1, 4), but relatively few cases have been reported where but two megaspores were produced, one disintegrating and one producing the nuclei of the megagametophyte. *Allium mutabile* is like *Chlorophytum orchitastrum* (5) so far as the megaspores are concerned. Its type of megaspore disintegration is that of type C of RUTGER'S second scheme, as given by SCHNARF (4). The one megaspore disintegrates as he shows, but unlike the one reported by SCHNARF, the first two nuclei in the megagametophyte are unequal. STRASBURGER (7) and SCHURHOFF (5) report two megaspores developing in *A. fistulosum* and *A. odorum*, the micropylar one disintegrating and the lower one dividing to form the megagametophyte. The variation between these and *A. mutabile* is not great. According to COULTER and CHAMBERLAIN, this would seem to indicate that the former two plants are as high in the plant kingdom as *A. mutabile*.

At maturity the megagametophyte contains only the essential cells. This condition would seem advantageous since this plant has a relatively short growing season. While four nucleate megagametophytes have been reported for *Clintonia* by SMITH (6), for *Cypripedium* by PACE (8), and for *Plumbagella* by DAHLGREN (2), the condition in *Allium mutabile* is actually different.

STRASBURGER (7) reports a megagametophyte for *Allium fistulosum* consisting of eight or nine nuclei, a large egg, unequal synergids, two polars, and several antipodals.

After the entrance of the pollen tube, the egg apparatus of *A. mutabile* superficially resembles that pictured by STRASBURGER, in that the large pollen tube lies in the position occupied by a synergid in his species. In this study the pollen tube was thus mistaken for a time.

The mature embryo compares essentially with that of *Allium cepa* (3), except for minor exceptions already mentioned. The notch develops in about the same region as reported by SCHNARF for *Luzula forsteri*. It develops lower on the embryo than he reports for *Poa annua*.

The thick walled nucellus is apparently uncommon; at least it has not been reported in accounts of other species of *Allium*. This condition probably accounts for the persistence of the nucellar cells, the thick cell walls being digested to provide food for the growing female nuclei. This variation, however, might be due to the conditions of drought which prevailed at the time the study was made.

Summary

1. In *Allium mutabile* two megaspores are produced; of these one disintegrates, while the other forms the megagametophyte.

2. The first division of the functional megaspore results in a large nucleus at the micropylar end and a smaller one at the antipodal end.

3. The larger nucleus divides twice to form the egg, one synergid, and two polar nuclei of the megagametophyte.

4. The smaller (antipodal) nucleus divides once to form two ephemeral antipodal nuclei.

5. At maturity the megagametophyte consists of a large vacuolated egg, a smaller synergid, and two still smaller polar nuclei.

6. The embryo develops normally and closely compares with those reported for other species of *Allium*.

7. Results of hand pollination show that both self and cross pollination are effective.

8. The cells of the nucellus are unusually heavy walled.

The writer wishes to express his appreciation to DR. ELDA R. WALKER for directing this study, and to others for their assistance.

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A FOSSIL ARAUCARIAN EMBRYO FROM THE CERRO CUADRADO OF PATAGONIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 476

BERTHA SCHWEITZER DARROW

(WITH THIRTEEN FIGURES)

Introduction

Although much fossil wood identified as Araucarian has been described, only a limited number of complete cones and isolated cone scales have been found. Dr. ELMER RIGGS of the Field Museum Patagonian Expedition (1924) discovered an extensive fossil forest at Cerro Cuadrado, Patagonia (13). More than one hundred excellently preserved cones were collected and these have made possible the study and description of the fossil embryo of at least one species. Dr. G. R. WIELAND has examined this material (13) and named the species *Proaraucaria mirabilis*. They have been referred both to the Triassic (13) and to the Lower Eocene (3).

The cones of two other species, *Araucarites windhauseni* (4) and *A. mirabilis* (12), were also found in Patagonia, in close proximity to the Cerro Cuadrado forest where Dr. RIGGS collected. Both of these species are regarded as identical with *Proaraucaria mirabilis* (Speg.) Wieland, but from the illustrations in the papers just mentioned, it seems that the Field Museum cones are in a far better state of preservation than any of those figured by either GOTHAN or SPEGAZZINI. The embryo is not referred to by GOTHAN, and SPEGAZZINI states that the *Araucarites mirabilis* cones were immature, embryos having not as yet developed when the cones were subjected to silicification.

With the exception of some foliage material of *Araucarites nathorsti* Dus. (2) from Punta Arenas on the Straits of Magellan, and two fragmentary cone scales of *Araucarites patagonica* (7) and *Araucaria* sp. (1), the fossils of the Cerro Cuadrado petrified forest (including the *Araucarites mirabilis* Speg. and the *A. windhauseni* Goth.) represent the only Araucarian fossils thus far described from South America.

SEWARD and FORD (10) discussed both the living and fossil Araucarians, and SEWARD (9) has reviewed all of the cone and foliage material described before 1919. GOTHAN (5) has since described a cone, *Conites araucarioides*, from the Tendagura of Africa, and another, *Araucarites obscurum* Wiel. (13), has been described from Como Bluff, Wyoming.

Methods

A preliminary study was made of all the *Proaraucaria mirabilis* cones in the Field Museum collection, especially those which Dr. WIELAND had cut and polished, in order to select for subsequent sectioning those showing exceptional preservation. Cone 13854 (Field Museum number) was finally chosen for detailed study because of its large size and because a tangential cut previously made by Dr. WIELAND revealed the presence of embryos.

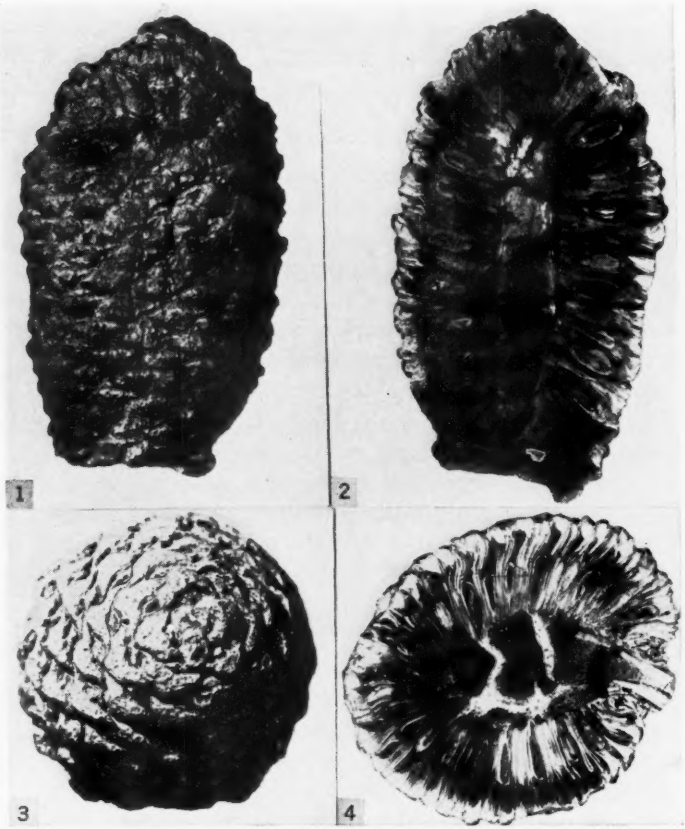
Ordinary methods for making petrological sections were used. Some thin sections were made, but in most cases polished slabs showed more detail than sections. A continuous series of transverse and longitudinal sections of the embryo of *Araucaria araucana* Koch., which grows at present in the Andes of Chile, not far from the locality of the fossil forest, was studied for comparison with the fossil specimens.

Description and discussion

CONE AND CONE SCALES.—Although one of the largest cones of the series of *Proaraucaria mirabilis*, number 13854 is small when compared with those of modern Araucarians, certain species of which have carpellate cones exceeding 30 cm. in length. The fossil cone (figs. 1, 2) measures 88 mm. in length and 50 mm. in diameter. The exterior is reddish brown. No tips of the cone scales were found. These may never have been present since other cones which show no erosion do not have a spine at the tip of the scale as do living species. The fossil may have been like the living *Araucaria angustifolia*, in which the spine falls off when the cone becomes mature. The ligule is conspicuous (fig. 3).

Internally the cone is composed of a jaspery silica of uniform, non-porous texture. The colors range from scattered spots of bril-

liant red, through black-brown, yellow, buff, milky blue, and white. Portions are colorless and transparent. There seems to be a marked



FIGS. 1–4.—Fig. 1, external view of half of *Proaraucaria mirabilis*, cone 13854; fig. 2, median longitudinal section of cone 13854; fig. 3, view looking down on apex of *P. mirabilis*, cone 13837; fig. 4, median longitudinal section of cone 13821; internal structure particularly well preserved.

tendency in this cone, as well as in others, for the various tissues of the seed and cone scale to have become impregnated in petrification with specific colors of quartz, almost as if the difference in tex-

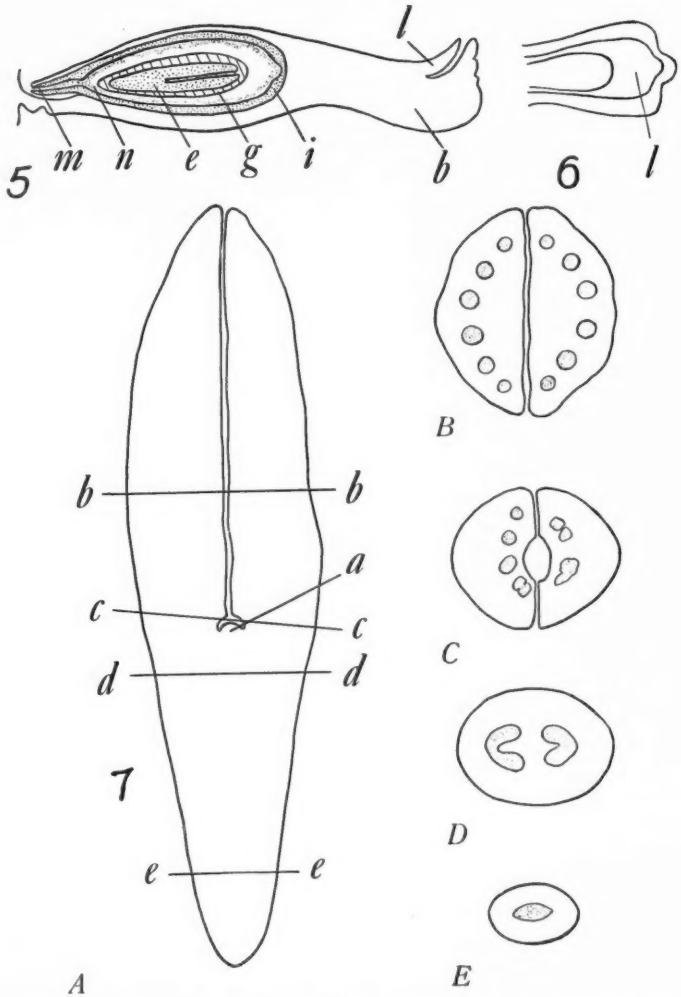
ture of the living determined the color of the fossilized tissues. For instance, the embryo is generally a light buff color, but in exceptional cases is a dark smoky, semi-opalescent brown. The vascular strands in the buff embryos are a light brown, so that they appear in good contrast to the general ground color. The megagametophyte is milky white, while the lumen formerly occupied by nucellar tissue is transparent. The remaining fragmentary nucellar tissue is milky white. The integument is always a light buff, and the other tissues of the cone scale are usually dark, but may be yellow, white, or a mixture of colors.

Cone 13821 of the Field Museum collection, being in general lighter in color, shows to better advantage this specific color distribution (fig. 4).

The pith of cone 13854 measures 10 mm. in diameter, and although it is smaller in this cone than in others of the collection, it is relatively large, as in modern Araucarian cones. It is bordered by a delicate cylinder of woody tissue. No pits could be distinguished on the tracheids. According to GOTHAN (**4**), the tracheids of wood associated with *Araucarites windhauseni* bear but a single row of closely spaced bordered pits, a situation not characteristically Araucarian.

The cone scales measure 20 mm. in length and are about 14 mm. broad at the distal end. They retain this width almost to the point of divergence from the cone axis, where they narrow to about 10 mm. Each bears in a median position a single totally imbedded seed, which occupies slightly more than the proximal half of the scale length. The cone scales differ from those described by GOTHAN (**4**) and SPEGAZZINI (**12**), in that these have prominent wings throughout their entire length (fig. 10, *B*).

The relative proportions of the cone scale are shown in figures 5 and 6. The structure parallels so closely that of the scales of modern Araucarians, especially those of the Eutacta section, that *Proaraucaria mirabilis* furnishes no added evidence to settle the controversy centered about the status of the carpellate cone scales in *Araucaria*. The structure of the vascular bundles of the scale also is not sufficiently well preserved to show their orientation, although the eroded apices of some of the scales show the presence of several vascular strands. Cell structure is unusually well preserved at the fleshy dis-



FIGS. 5–7.—Fig. 5, diagram of median longitudinal section of cone scale (*b*, bract; *l*, ligule; *i*, integument; *g*, megagametophyte; *e*, embryo; *n*, nucellus; *m*, micropyle). Fig. 6, surface view from above of cone scale. Fig. 7, *A*, median longitudinal section of embryo showing cotyledons and hypocotyl; stem tip indicated at *a*. *B*, *C*, *D*, *E*, transverse sections of embryo showing vascular structure taken at planes *b–b*, *c–c*, *d–d*, and *e–e* on longitudinal diagram, fig. 7 *A*.

tal end of the scale. Adaxially from the chalazal end of the seed, however, the preservation of the cell structure of the interior tissues of the scale fails, only an exterior border four or five cells deep being present. The heavy walls indicate that the scale may have been woody.

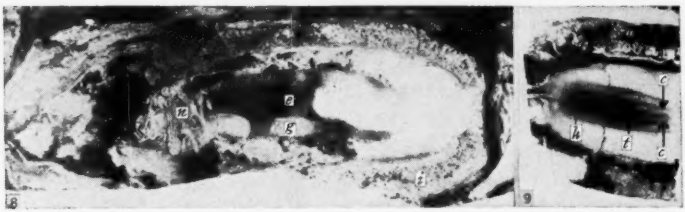
Because most of the recorded fossil Araucarian cone material consists of isolated cone scales lacking associated leaf material, it has little value for comparison with the present fossil. If the cone scales of *Proaraucaria mirabilis* had been found isolated, they would undoubtedly be classified in the Eutacta section of the genus on account of their broad wings and prominent ligules. The association with the cones of twigs bearing the broad leaf scars of the Colymbea type, however, as well as the fact that the embryo has two cotyledons, raises the question of their exact affinity. *Araucarites microphylla* Saporta, from the Jurassic of France (**8**), has in association with its Eutacta type scales, leaves of the Colymbea type, similar to those of *Araucaria bidwillii* Hook. but smaller. The North American *Araucarites hespera* Wiel. (**14**), which has Eutacta scales, has been found associated with *Araucarites hatcheri* Wiel. (**14**) which has Colymbea type leaves, and may represent a second example of Eutacta scales and Colymbea leaves in the same plant.

Among modern forms the cone scales of *Proaraucaria mirabilis* may be most closely compared with those of *Araucaria bidwillii*, which have broadly winged scales bearing prominent ligules, Colymbea leaves, and an embryo having two cotyledons. The latter is regarded as a transitional form between the Eutacta and the Colymbea sections, but is classified as a Colymbea form because of its leaves, its two cotyledons, and the hypogeal germination of its seeds. The points of difference from *Proaraucaria mirabilis* are its much larger cone size and the presence of a long spine at the scale tip.

The two living South American species, *Araucaria angustifolia* (Bertol.) Ktze. and *A. araucana* Koch., are characterized by having much reduced scale wings, the former species having none at all. In addition, both have relatively inconspicuous ligules, Colymbea leaves, two cotyledons, and hypogeal germination. This reduction in the modern South American species may well represent development from types such as *Proaraucaria mirabilis*, which were present

and dominant in the same geographic locality in past ages, specialization accompanied by isolation finally resulting in the modern type. Curiously enough, it is only the South American species which exhibit this reduction in scale wings. Also, this seems to be a comparatively recent development, as none of the fossil scales found in any part of the world are of this reduced type.

SEED.—The seeds of cone 13854 average 11 mm. in length. They are somewhat flattened, so that the diameter parallel to the scale measures about 6 mm. at the broadest region, while the diameter at right angles to the scale seldom exceeds 4 mm. The integument



FIGS. 8, 9.—Fig. 8, longitudinal section of seed, cut so as to remove one cotyledon of embryo (*i*, integument showing thin area at chalazal region; *n*, nucellus showing wavy nature as if disintegrating or crushed; *g*, megagametophyte; *e*, embryo). Fig. 9, median longitudinal section of embryo showing both cotyledons (*c*), stem tip (*t*), and hypocotyl (*h*). Tips of cotyledons have been removed. Vascular strands may be traced from cotyledons to hypocotyl.

seems to be composed of three layers, an outer and inner thin layer and a much heavier, fibrous, middle layer. The thick middle layer thins abruptly at the chalazal region. Closely appressed to the inner surface of the seed coat is a thin layer of nucellar tissue. It must have shrunken markedly before petrification, leaving a lumen which was subsequently filled with transparent quartz. At the micropylar end of the seed the nucellar tissue is more abundant, and extends into the micropylar tube. Here it has a wavy appearance, as if the living tissue had been partially digested or crushed.

The tissue of the megagametophyte has been replaced by a dense ivory colored silica, devoid of cell structure. It is massive, measuring 2.5 to 3 mm. by 1 to 1.5 mm. in cross section, and having a length of 5.5 to 6 mm.

Notwithstanding their small size, about 5 mm. in length and only

1 mm. in diameter, the embryos are mature or approximately so. They extend almost the full length of the megagametophyte, and have two well defined cotyledons and a well developed hypocotyl.

In the median longitudinal section, represented diagrammatically in figure 7 *A*, the cotyledons are seen to diverge slightly below the middle of the embryo. The relatively flat stem tip is indicated at *a*.

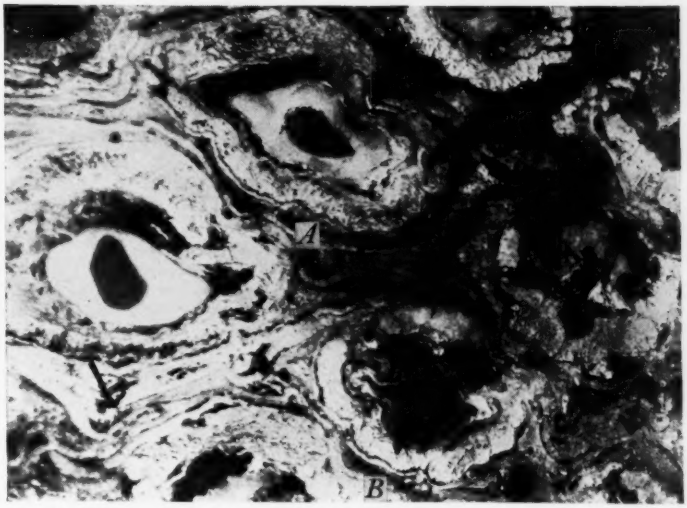
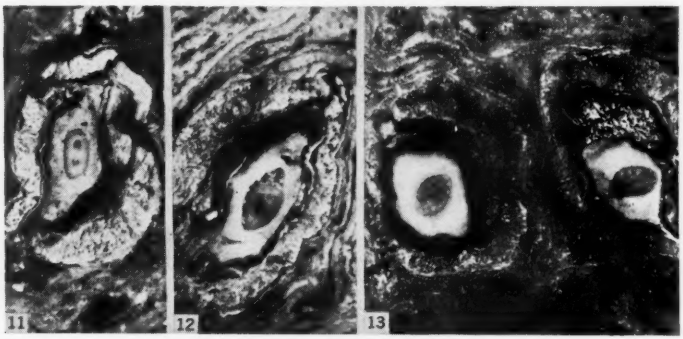


FIG. 10.—Transverse sections of several seeds cut at various planes. Section at *A* cuts embryo near base of hypocotyl, showing diarch stele. Seed at *B* cut through nucellar region below base of hypocotyl near to cone axis. Tips of the broad scale wings at this adaxial location are indicated by the two arrows.

B, *C*, *D*, and *E* of figure 7 represent diagrams of transverse sections taken at *b*–*b*, *c*–*c*, *d*–*d*, and *e*–*e* respectively on the longitudinal diagram. The vascular pattern is as follows: At the base of the hypocotyl (*E*) the stele is diarch, and is represented by a lens-shaped centrally located mass. Farther up, at *D*, which represents a section slightly above the cotyledonary plate, the vascular strands are grouped in two crescent-shaped masses. At *C* the section passes through the embryo just above the stem tip, as is indicated by the presence of the circular opening at the center. Here the two cotyledons are distinct and the vascular bundles of the cotyledons are

distinguishable. The plane of the cut in *C* was not exactly at right angles to the perpendicular axis of the embryo, so that the cotyledon on the left has been cut at a slightly higher level than that on the right, and consequently shows a greater number of bundles. The cross section at *B* is at a more distal plane in the cotyledons, where the full number of six bundles in each cotyledon is distinct.

Since the diamond saw removes with each cut more than 1.5 mm. of material, it is obvious that not enough serial sections could be



FIGS. 11–13.—Fig. 11, transverse section of seed showing embryo cut through region of cotyledonary plate. Vascular tissue represented by two crescent-shaped masses. Numerous resin ducts visible below epidermal region. Fig. 12, embryo cut just above point of divergence of cotyledons. Space just above stem tip shown at center. Cotyledon above shows presence of four bundles, lateral pair on point of diverging to form a total of six. Cotyledon below shows two masses of vascular tissue, each about to divide. Resin ducts may also be seen below epidermal region. Fig. 13, transverse section of two seeds cut at more distal plane of embryo; two cotyledons distinct in both embryos, each having six vascular strands. Resin ducts also visible.

obtained from any single embryo to supply all the data necessary for the reconstruction. Figure 7 therefore represents a hypothetical reconstruction from observations made on several embryos.

The embryo of *Proaraucaria mirabilis* is in general very similar to those of modern species of the Colymbea section of the genus *Araucaria*, both in exterior aspect and in vascular anatomy. In the modern species, *Araucaria araucana* and *A. angustifolia*, the hypocotyl constitutes less than one-sixth of the total length of the embryo, while in the fossil it comprises about one-half.

The vascular pattern corresponds closely with that of *Araucaria*

araucana. A minor difference is that the number of cotyledonary bundles in the modern form is variable, ranging from six to eight, while never more than six were observed in the fossil. The vascular structure also resembles closely that of *A. angustifolia* discussed by HILL and DE FRAINE (**6**), and *A. bidwillii* given by SHAW (**11**).

Summary

1. The cones of *Proaraucaria mirabilis* show the most excellent preservation of any fossil *Araucaria* cones thus far recorded.

2. Associated with them are other portions of the plant, including twigs bearing the broad leaf scars typical of Colymbea leaves.

3. The cone scales have the characteristics of those of the Eutacta section of the genus *Araucaria*, that is, broad scale wings and prominent ligules, but the fossil is considered a Colymbea form because of its broad leaves and because its embryos have two cotyledons.

4. *Proaraucaria mirabilis* thus resembles the fossils *Araucarites microphylla* Sap. and *A. hespera* Wiel., which had Eutacta cone scales and Colymbea leaves.

5. *Proaraucaria mirabilis* is to be most closely compared with *Araucaria bidwillii*, the only living species which combines winged scales with broad Colymbea leaves. The cones of *A. bidwillii*, however, are much larger than those of the fossil.

6. The embryos of *Proaraucaria mirabilis* are in general similar, although much smaller than those of living species of the Colymbea section. Their chief difference, aside from their small size, is that they have a proportionately much larger hypocotyl than the living forms.

7. The gross vascular anatomy corresponds closely to that in *Araucaria araucana*, *A. angustifolia*, and *A. bidwillii*.

The writer expresses appreciation to Dr. A. C. NOÉ, under whose direction the study was conducted, and to the Field Museum of Natural History for lending the specimens for study. Dr. G. W. GRAVES of State Teachers College, Fresno, California, Dr. L. L. BURLINGAME of Leland Stanford University, and Dr. P. F. SOUSA of Rio de Janeiro kindly furnished seed and cone material of living Araucarians.

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MORPHOLOGICAL AND CULTURAL STUDIES OF TAPHRINA POTENTILLAE[1]

ELLA MAY MARTIN

(WITH ELEVEN FIGURES)

Introduction

Early descriptions of *Taphrina potentillae* Farw. Johanson give briefly the morphology of the fungus and the symptoms shown by the host, *Potentilla canadensis*, but apparently there has been no report of cultural or inoculation experiments with this fungus. FARLOW (2), JOHANSON (6), and ROBINSON (14) measured and described the ascus. JUEL (7) described and figured the ascogenous cell and the young ascus.

Morphological studies

MATERIALS AND METHODS.—Leaves of *Potentilla canadensis* infected with *Taphrina potentillae* were collected and fixed early in July, 1928, near Ithaca, New York, and the following May at Greensboro, North Carolina. At Greensboro, plants had been successfully inoculated with cultures isolated at Ithaca. Gilson's and Flemming's weaker fixing solutions proved to be the best for a study of the fungus. Material was dropped into Carnoy's solution for one minute before it was placed in the other fixing solutions. This method gave more satisfactory results than did the use of an air pump. By fixing material in the field immediately after collection there was the least plasmolysis. Paraffin sections were cut from 2 to 5 μ in thickness. They were stained with Flemming's triple stain and by the anilin oil gentian violet method as used by Claussen.

SYMPTOMS AND EFFECTS ON HOST.—Circular or elongated blisters are produced on the leaflets of *Potentilla canadensis* infected with *Taphrina potentillae*. The infected areas become convex on the upper surface and concave on the lower, and vary in length from 1 mm. in

[1] A part of this paper was presented before the American Phytopathological Society at Cleveland in 1930; an abstract was published under the title: Cultural and inoculation experiments with *Taphrina potentillae*. Phytopath. 21:121. 1931.

their early stages to 1 cm. when older. When first noticeable they are yellow, but later they become purple and brown. Lesions are most frequent near the lateral margins and tips of the leaflets (fig. 1), but some are located near their bases. Swelling of the petiole described by JOHANSON, when found in this material, is very slight.

The leaflets are not distorted by this fungus so much as are the leaves of the peach when infected with *Taphrina deformans*. In *Potentilla canadensis*, the diseased areas, which are only small blisters, are thicker than normal parts. This thickening and blistering is due to an increase in the number of cells in the palisade and upper portions of the spongy parenchyma.

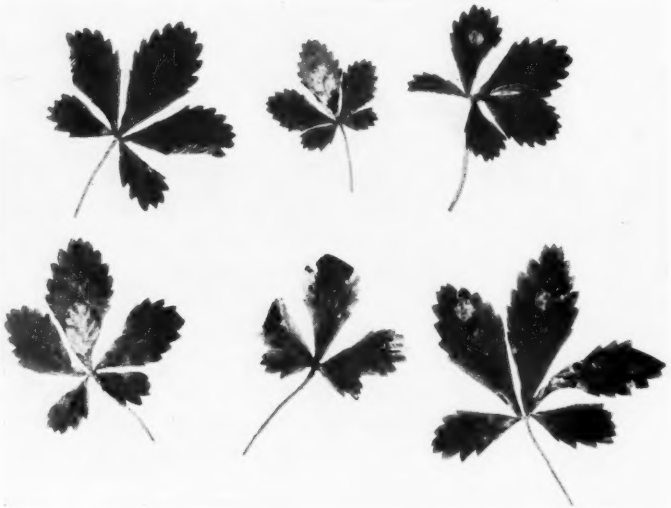


FIG. 1.—Typical lesions produced by *Taphrina potentillae* on leaves of *Potentilla canadensis;* each leaf inoculated with a different combination of single spore cultures.

MORPHOLOGY OF FUNGUS.—The form of the vegetative and the ascogenous cells of *Taphrina potentillae* is largely determined by the host cells surrounding them. The vegetative cells may vary from ovoid to cylindrical as they grow between the mesophyll cells of the leaf. Both the vegetative and the young ascogenous cells are binucleate (figs. 2–4). The latter are frequently ovoid and are scattered

irregularly beneath the epidermal layer. Their position beneath the epidermis was early mentioned by GIESENHAGEN (4) and SADEBECK (15) for this species of *Taphrina* and for *T. flava*. In all other species of *Taphrina* the ascogenous cells form a definite layer between the cuticle and the epidermis.

As the ascogenous cells grow between the epidermal cells to form asci, they become very slender, but after they have broken the cuticle and have grown beyond the surface of the leaf, they broaden considerably. Thus the asci which are found on both surfaces of the leaf are clavate, with their pedicels remaining between the epidermal cells.

As the ascogenous cell develops into an ascus, the two nuclei in it fuse (fig. 5), and the fusion nucleus, according to JUEL, soon undergoes division. Two successive mitotic divisions have been described by JUEL for this species. It is assumed that three successive divisions occur in the developing ascus, thus forming the eight nuclei for the primary ascospores.

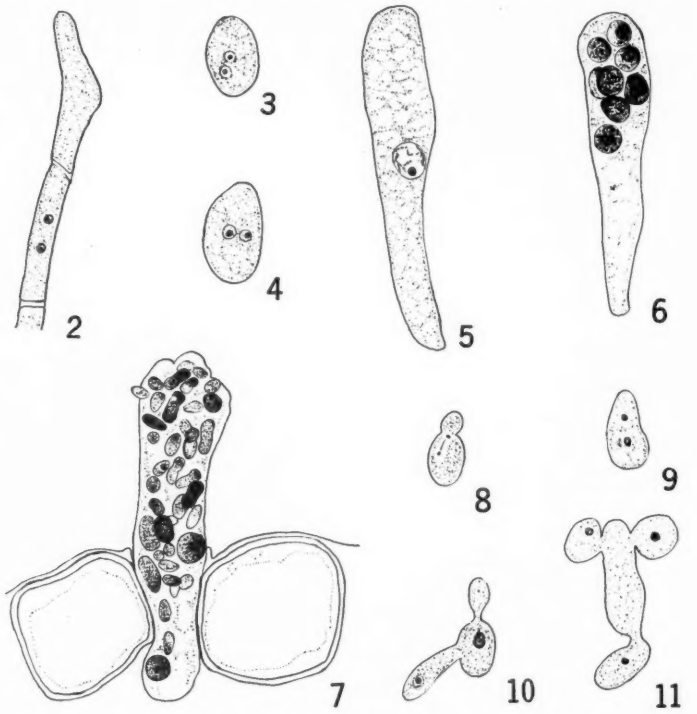
Eight primary ascospores are commonly found in the ascus (fig. 6), but before the spores are discharged budding takes place, resulting in a large number of minute spores in the ascus (fig. 7). Occasionally one of the large ascospores may continue without budding up to the time of spore discharge. Ejection of the spores occurs at the apex of the ascus, leaving an irregularly broken margin.

Cultural studies

METHODS OF ISOLATION.—Diseased portions of leaves were fastened to the covers of petri dishes so that the asci could discharge their spores upon the agar below. After one-half hour, the petri dishes were inverted and individual spores were marked by using an 8 mm. objective and a 12 or 15× ocular. The media used were clear Harter agar made without peptone, potato dextrose agar, and a dextrose agar to which juice from *Potentilla* leaves was added. The pH of these agars ranged from 4.5 to 5.

Spores of this species of *Taphrina* are extremely small when they are discharged, their dimensions often averaging 2.5 × 3μ. Their transparency also makes the marking of them a tedious process. This difficulty was overcome by using a clear potato dextrose agar

to each liter of which 0.1 gm. of Congo Red had been added. Congo Red is readily absorbed by *Taphrina* spores but does not hinder their growth. The petri dishes were next placed in a constant temperature



FIGS. 2–11.—Fig. 2, vegetative mycelium of *Taphrina potentillae* within leaf of *Potentilla canadensis;* figs. 3, 4, binucleate ascogenous cells; fig. 5, young ascus containing fusion nucleus; fig. 6, eight spored ascus; fig. 7, many spored ascus; figs. 8–11, stages in budding of spores on Sabouraud's agar.

chamber at 9° C. for twelve hours or a little longer. It had been found previously with *Taphrina coryli* (9) that chilling the spores for twelve hours and then bringing them up to room temperature would hasten germination. Spores germinated rapidly after one-half hour at room temperature, and having absorbed Congo Red, were easily marked. Only the more isolated of these germinating spores were

marked, and colonies developing from them were transferred to potato dextrose agar slants. Thus twenty-eight single spore cultures of *T. potentillae* were obtained. In cases where spores were not well scattered, dilution plates were poured. The color absorbed from the Congo Red was retained by the organism for some time, but after several transfers to standard potato dextrose agar without Congo Red, the cultures became pale pink in color and similar to those which were isolated on ordinary potato dextrose agar. The latter cultures are a grayish pink, opaque, and glistening, and are very much like agar cultures of *T. johansonii* described by the writer (10) and *T. deformans* sent to the writer by MIX and described by him (12). Compared with the colors given in Ridgway's Color Standards and Color Nomenclature, cultures of *T. potentillae* might be described as "pale vinaceous fawn."

INOCULATION EXPERIMENTS.—The agar cultures which were isolated at Ithaca were taken to Greensboro, where they were used the following spring for inoculating plants of *Potentilla canadensis*. No infected plants had been found in that region and none had been reported nearer than Durham, North Carolina, 50 miles away. The plants selected for experimental work were growing 4 to 6 feet apart. Since the hillside on which they grew was burned over each February, all plants available for inoculation had leaves of only the current season's growth.

Inoculations were always carried on in the late afternoon during a light rain. Young leaves were selected on each plant and a small amount of potato dextrose agar with the spores was spread on the upper and lower surfaces of each leaflet. Before and after inoculating, the plants were sprayed with sterile distilled water. Plants were covered with fine meshed cheesecloth or muslin well elevated over a framework.

Following the method just described, thirty-eight plants were inoculated March 26 and 27, 1929. Eleven single spore cultures were selected for use. Assuming that these cultures might be plus or minus, five of them were used alone and these and the others in different combinations. By May 11 of that year, 50 per cent of the plants showed characteristic blisters. The amount of infection was the same whether plants were inoculated with one culture or with a

combination of cultures. Seventeen of the thirty-three plants inoculated with combinations of cultures and two of five plants inoculated with single spore cultures became infected. The lesions on leaves shown in figure 1 were produced by combining different cultures.

The next spring, eight of the same cultures were used in inoculating sixteen other plants which were located a quarter of a mile away from the plants used previously. This time 75 per cent of the plants showed infection even though eight of them were inoculated with single spore cultures and the other eight with combinations of cultures. Six of each group of plants showed the blisters characteristic of plants infected by *Taphrina potentillae*.

Plants infected as a result of inoculations during these two years did not show infections in later years. Since the areas where they were growing were burned over each February, spores were evidently killed, for the new leaves coming from the rhizome were not infected.

Climatic conditions evidently have much influence upon the amount of infection resulting from inoculation. Plants inoculated in the autumn with the same cultures showed no infection although some were grown all winter out of doors under muslin and others were kept in the laboratory covered with bell jars. Negative results were also obtained with leaves inoculated at the same time and kept in moist sterile petri dishes.

SPORE GERMINATION AND CONJUGATION.—Spores of *Taphrina potentillae*, when discharged from the ascus, continue to carry on their yeast-like budding, either on the surface of leaves of the host or on the media mentioned.

All workers are in agreement on the budding of ascospores of *Taphrina* species, but there are decided differences of opinion as to whether conjugation takes place. KLEBAHN (**8**) and WIEBEN (**16**) described conjugation for some species of *Taphrina*. In these species they hold that the two nuclei present in the cells of the vegetative mycelium result from the conjugation of spores or of young hyphae. JANKOWSKA (**5**), however, working with *T. sadebeckii*, found no conjugation. The writer (**11**) reported that spores of *T. johansonii* and *T. deformans* did not conjugate on artificial media. FITZPATRICK (**3**)

saw no cases of conjugation in *T. deformans*, but MIX (12) found occasional, although incomplete, conjugation in the same species.

With these results in mind, the first work done by the writer was to grow single spore cultures and combinations of these cultures, as reported in 1931 (footnote 1). The cultures were grown in Van Tieghem cells in drops of the following media: cleared potato dextrose agar, Sabouraud's agar, a decoction made from *Potentilla* leaves, and a dextrose agar made with the juice from *Potentilla* leaves. The hydrogen-ion concentration of these types of media was from 5.4 to 5.9. This material was fixed in Juel's or in Bouin's fixing solution, stained with iron-alum haematoxylin or Flemming's triple stain, and mounted in Venetian turpentine or balsam. Similar material was removed from inoculated leaves and treated in the same manner.

The slides made previous to the report in 1931 were re-examined during the present year. It is now seen that cases which appeared to be conjugation (figs. 10, 11) are rare, and in no case have the spores which are in contact shown a complete movement of the protoplast from one spore to the other. Budding, however, is abundant and is always preceded by nuclear division (figs. 8, 9). This nuclear division in the budding spore produces the binucleate condition of the young mycelium.

Discussion

Spores and young hyphae which appear to be conjugating are found so seldom that they cannot account for the presence of two nuclei in the cells of the young mycelium. Such apparent conjugation may represent only irregularities in budding, or chance contact of spores; or in young germ tubes it may be merely the anastomosing of hyphae. The latter relationship, if it does exist, evidently does not constitute a definite part of the life cycle of the organism.

From these investigations, showing that either single spore cultures or combinations of them may produce infection, it is assumed that *Taphrina potentillae* is a homothallic form. In this species the dicaryophase is evidently initiated by division of the nucleus in the spore at the time of germination, as suggested by EFTIMIU (1) and FITZPATRICK (3) for *Taphrina deformans*, and by the writer (11) for this species and *T. johansonii*.

Summary

1. *Taphrina potentillae* produces circular or elongated blisters on the leaflets of *Potentilla canadensis*. The ascogenous cells of this fungus are produced between the epidermis and the mesophyll, but they grow between and beyond the epidermal cells to form asci with slender pedicels. Eight primary spores are formed in the ascus but these bud before they are discharged, forming numerous minute spores.

2. Spores isolated on potato dextrose agar form colonies which are grayish pink, opaque, and glistening.

3. Plants inoculated either with combinations of the eleven single spore cultures or with only single spore cultures showed infection.

4. Stages which appear like conjugation of spores are rare and do not represent the union of protoplasts, so this is not now considered conjugation.

5. Spores of *Taphrina potentillae* bud to form short chains and then hyphae, either on artificial media or on the surface of the host. Nuclear division in the budding ascospore initiates the dicaryophase.

This study was completed while the writer had guest privileges in the Department of Botany at the University of Chicago.

UNIVERSITY OF CHICAGO

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CYTOLOGICAL INVESTIGATIONS OF ERECHTITES HIERACIFOLIA

GEORGE OLDS COOPER

(WITH THIRTY-SEVEN FIGURES)

Introduction

Aside from the single genus *Senecio*, few cytological investigations have been made in the Senecioneae. GUIGNARD (7) briefly described the mature embryo sacs of *Petasites* and *Tussilago*. AFZELIUS (1), CARANO (4), DAHLGREN (5), and SMALL (8) found a normal type of development for the megagametophyte in *Senecio* but laid major emphasis on the behavior of the antipodal cells. AFZELIUS showed a multinucleate condition in the antipodals of *Cacalia suaveolens* and *C. reformis*. SMALL found a similar condition in *Tussilago* and *Petasites*. He likewise described microsporogenesis and the development of the anther in *Senecio vulgaris*. SOUÈGES made a detailed study of the development of the embryo in this species.

Material and methods

The material of *Erechtites hieracifolia* (L.) Raf. was gathered on the campus of the University of Wisconsin. The investigation was carried on through the facilities of the Departments of Botany and Genetics. Since the flowers are produced in a slowly maturing head, buds were collected at various stages of development. They were fixed either in Carnoy's solution or in Karpechenko's modification of Navashin's solution, imbedded in paraffin, and cut 12–14 μ in thickness. They were stained either in Delafield's haematoxylin or in Smith's modification of Gram's iodine-crystal-violet. The latter was found to give better differentiation in material at early stages of development. All drawings were made with an Abbé camera lucida at table level.

Investigation

MICROSPOROGENESIS.—Each theca of the anther contains a single row of microspore mother cells which are considerably larger than the surrounding vegetative cells. From five to seven such cells are found in each row (fig. 1). As the microspore mother cells approach

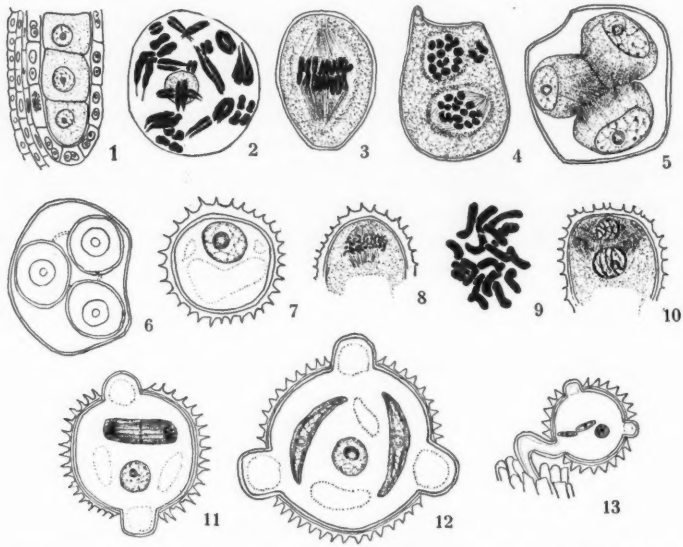
synizesis, mitotic divisions occur in the tapetal cells so that each cell is binucleate at maturity. At diakinesis 20 pairs of chromosomes are present (fig. 2). AFZELIUS has reported the same number for *Senecio vulgaris*, whereas SMALL found 19 to be the gametic number. A marked enlargement of the microspore mother cell occurs during the period in which the nucleus is passing through the late prophase stages. At metaphase of the heterotypic division (fig. 3) each of the chromosomes is split longitudinally. The spindles of the homoeotypic division are usually at right angles to each other (fig. 4), but occasional cells were found wherein they were parallel. Twenty chromosomes were present at the homoeotypic division (fig. 4). A somewhat denser zone of cytoplasm surrounds the spindles of both the heterotypic and homoeotypic divisions (figs. 3, 4). These were particularly pronounced at the homoeotypic equatorial plate stage. The second peripheral zone of cytoplasm observed by D. C. COOPER (2) in *Portulaca oleracea* and by the writer (3) in the Chenopodiaceae was not observed.

As a result of the homoeotypic division, a cell containing four nuclei is formed. The spindles of the meiotic divisions persist for a time and cell plates are laid down across their mid-regions (fig. 5). In the process of spore formation splits occur along these cell plates, an observation which is at variance with that of GATES and REES (6) who state the spores are formed as a result of furrowing.

The newly formed microspores are held together for a time in a matrix of stainable material. The microspore increases in size and an irregular spiny exine is developed (fig. 7). The intine appears shortly after the division of the microspore to form a two celled gametophyte.

The microspore nucleus divides to form the generative nucleus and the tube nucleus (fig. 8). A cell plate is laid down across the spindle and the microspore is divided to form a large tube cell and a smaller generative one (fig. 10). The method of procedure is similar to that noted by the writer as occurring in the Chenopodiaceae. The elongated generative cell ultimately becomes deeply imbedded in the cytoplasm of the tube cell and its limiting membrane is scarcely perceptible except in thin sections. Shortly before anthesis the generative cell divides to form two male gametes. The nuclear division is typically mitotic and at telophase a cell plate is formed across the

mid-region of the spindle (fig. 11). The two male gametes exist as distinct cells which become much elongated and sickle-shaped as they approach maturity (fig. 12). Each pollen grain has three germ pores more or less equally spaced on its surface (fig. 12). Each of these pores contains a large vacuole and is covered with a thin intine.



FIGS. 1–13.—Microsporogenesis: fig. 1, longitudinal section through anther showing single row of spore mother cells; fig. 2, diakinesis, 20 pairs of chromosomes; fig. 3, heterotypic metaphase; fig. 4, homoeotypic metaphase, spindles at right angles; fig. 5, cytokinesis, thickenings on spindles and partition walls beginning to advance across spindle; fig. 6, four spores in matrix of old microspore mother cell; fig. 7, mature microspore; figs. 8–10, stages in division of microspore nucleus; fig. 11, telophase in division of generative cell; fig. 12, mature pollen grain; fig. 13, germinating pollen grain.

The exine is lacking in this region. Shortly after the pollen comes in contact with the stigmatic surface, the pollen tube emerges at one of the germ pores and passes between the cells of the stigma (fig. 13). The male gametes and the tube nucleus remain in the pollen grain for some time after the formation of the pollen tube. Ultimately they likewise pass into the pollen tube and along it to the micropylar end of the megagametophyte.

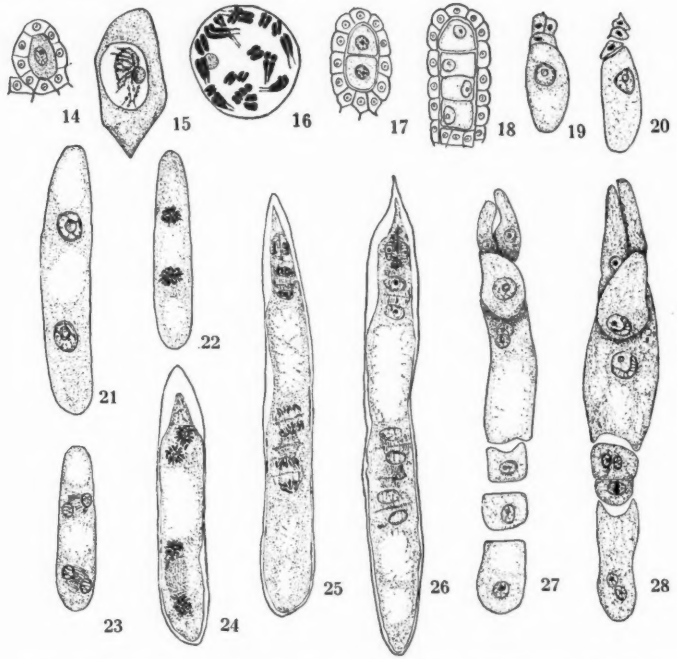
MEGASPOROGENESIS.—The single archesporial cell is hypodermal

and functions directly as the megaspore mother cell (fig. 14). During the process of the development of the ovule the megaspore mother cell grows both in length and width. Its nucleus likewise enlarges while passing through the stages leading to the diakinesis stage of the heterotypic division. The cytoplasm of the cell remains finely vacuolate. Twenty pairs of chromosomes are present at diakinesis (fig. 16). Following the heterotypic division two cells are formed (fig. 17), and subsequent to the homoeotypic division a linear row of four megaspores (fig. 18) results. Shortly after the formation of the megaspores, the chalazal one elongates and the other three disintegrate (figs. 19, 20). SMALL likewise found that the basal cell functions in *Senecio vulgaris* and that the three micropylar spores disintegrate.

The functional megaspore nucleus divides to give a two nucleate megagametophyte (fig. 21). A large vacuole develops in the midregion of the cell between the two nuclei. As a result of the two further divisions an eight nucleate megagametophyte is formed. In the course of the last division of the nuclei, the spindles of the two chalazal nuclei are end to end so that a linear row of four is formed. The two spindles at the micropylar end are at right angles to each other, the longitudinal axis of the apical spindle being transverse to the longitudinal axis of the megagametophyte. Cell plates are formed across the spindles of the last division as well as across the persistent spindles of the preceding division in such a manner as to form three uninucleate cells at each end of the megagametophyte, and the polar nuclei one from each end remain in the large central cell (figs. 25, 26). The completed eight nucleate, seven celled megagametophyte consists of a linear row of three antipodals, a primary endosperm cell containing two nuclei, a pear-shaped egg cell and two club-shaped synergids. The basal antipodal cell is about twice as large as either of the other two (fig. 27). The cytoplasm of the synergids is finely vacuolate in the basal region near the egg cell and elongate striations occur in the apical region. The cytoplasm of the megagamete is finely vacuolate until just prior to fertilization.

Double fertilization occurs, one male gamete uniting with the primary endosperm nucleus formed as a result of the union of the two polar nuclei, the other uniting with the egg nucleus (fig. 28).

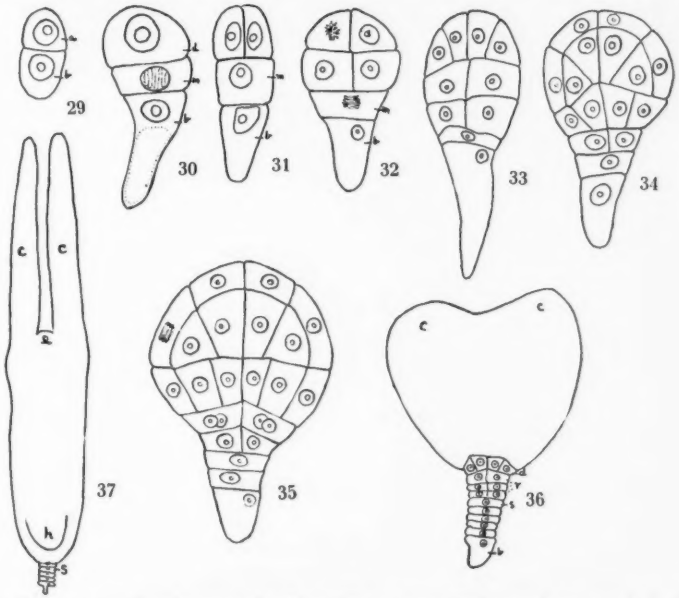
The cytoplasm of the egg cell develops elongate vacuoles in the apical region. The antipodal cells are usually binucleate at the time of fertilization.



FIGS. 14–28.—Megasporogenesis: fig. 14, megaspore mother cell; fig. 15, same, nucleus at synizesis; fig. 16, diakinesis, 20 pairs of chromosomes; fig. 17, interkinesis; fig. 18, linear row of four megaspores surrounded by nucellus; figs. 19, 20, functional megaspore with three disintegrating spores; figs. 21–28, stages in development of megagametophyte: fig. 21, two nucleate stage; fig. 22, metaphase preceding four nucleate stage; fig. 23, early four nucleate stage; fig. 24, metaphase preceding eight nucleate stage; figs. 25, 26, cell plate formation; fig. 27, mature megagametophyte, uninucleate antipodals; fig. 28, double fertilization, antipodals binucleate.

In some instances the antipodals remained uninucleate even after fertilization, and in no instance was a multinucleate antipodal observed. AFZELIUS found the antipodals of the forms he studied to be either binucleate or multinucleate. He states that in some instances the antipodals may divide so that instead of having typically three

antipodal cells, a larger number may be present. SMALL records that the chalazal antipodal may divide to form as many as four cells, each cell having one or more nuclei. This elongated structure is spoken of as an aggressive haustorium. Such variations in antipodal formation were not observed in *Erechtites*.



FIGS. 29–37.—Development of embryo: Fig. 29, two celled proembryo; *a*, apical cell; *b*, basal cell. Fig. 30, three celled proembryo; *a'*, apical cell; *m*, middle cell; *b*, basal cell. Fig. 31, two celled embryo with two celled suspensor. Fig. 32, four celled embryo, middle cell of suspensor dividing. Figs. 33–35, further development of embryo. Fig. 36, first evidences of cotyledons, *c;* dermatogen, *d;* root cap, *r*. Fig. 37, mature embryo; *e*, epicotyl; *h*, hypocotyl; *s*, suspensor.

DEVELOPMENT OF EMBRYO.—The zygote divides to form a two celled proembryo with cells about equal in size (fig. 29). The apical cell divides so that a row of three cells is formed (fig. 30), consisting of an elongated basal cell (*b*), a somewhat flattened middle cell (*m*), and a rounded terminal cell (*a'*). The terminal cell becomes greatly enlarged and then divides vertically to form the two celled embryo at the apex of a two celled suspensor (fig. 31). Later a transverse di-

vision of these two cells produces a four celled embryo (fig. 32). In this development *Erechtites* differs from *Senecio* wherein the middle cell of the three celled stage divides first and the distinct quadrant of cells is lacking (**9**). Further development of the embryo is comparable in the two genera. Periclinal divisions of the quadrant cut off the dermatogen. Later the periblem and plerome are delimited and in the process of further differentiation the embryo becomes spherical (figs. 34, 35). As a result of active cell division the embryo elongates and becomes flattened at the apex. Shortly thereafter the primordia of the cotyledons appear. This is in agreement with that shown in other Compositae. The basal and suspensor cells now show signs of disintegration. The cotyledons elongate further, the epicotyl appears, and the cyclic arrangement of the cells at the base of the elongating hypocotyl shows the beginning of the root cap (fig. 37).

Summary

1. Each theca of the anther of *Erechtites hieracifolia* contains a single row of five to seven microspore mother cells.

2. The heterotypic and homoeotypic spindles are surrounded by a dense sheath of granular cytoplasm.

3. Paired thickenings appear on the cytoplasmic strands midway between the nuclei of the tetrad, and the four spores are formed as the result of these cell plates.

4. The spore divides to form a two celled pollen grain.

5. The generative cell becomes deeply imbedded in the cytoplasm of the tube cell and then divides to form two male gametes.

6. A single megaspore mother cell is found in the ovule of each flower.

7. As a result of meiosis a row of four megaspores is formed.

8. The chalazal megaspore develops into an eight nucleate, seven celled megagametophyte, and the other megaspores disintegrate.

9. Double fertilization occurs.

10. At the time of fertilization the antipodals are usually binucleate.

11. A proembryo of three cells is formed.

12. The embryo arises as a result of further divisions of the apical cell of the proembryo.

13. The suspensor is formed from the two basal cells of the proembryo.

14. The haploid chromosome number is 20.

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CYTOLOGY OF CERTAIN LABIATAE

ELIZABETH P. BUSHNELL

(WITH TWENTY-TWO FIGURES)

Only a few genera of Labiatae have been investigated from the standpoint of chromosome number and chromosome morphology. The haploid numbers in *Galeopsis* are reported by MÜNTZING (3) to be eight and sixteen. JÖRGENSEN (2) made chromosome counts of ten species of *Lamium*, seven of which had the haploid chromosome number of nine; in the others the number was eighteen. SCHEEL (6) determined the chromosome numbers in species of *Rosmarinus*, *Scutellaria*, *Lavandula*, *Sideritis*, *Coleus*, and *Salvia*. RUTTLE (4) found two species of *Mentha*, *M. requienii* with nine chromosome pairs and *M. pulegium* with ten, that differ in morphological character as well as in chromosome number from other members of the genus. Other species of *Meniha* form a twelve-chromosome series. The diploid number in three species of *Lycopus* RUTTLE (5) determined to be twenty-two. HRUBY (1) reported sixteen as the haploid number in three species of *Prunella*.

For the present study, flower buds of *Monarda fistulosa*, *M. didyma*, *M. punctata*, and *Nepeta cataria* were fixed with Navashin's, Licent's, and Flemming's fluids. The material was sectioned at 10–15 μ; sections 13 μ in thickness included whole nuclei and were most valuable for study. Stains used were Newton's iodine-crystal violet, Flemming's triple, and Heidenhain's iron-alum haematoxylin.

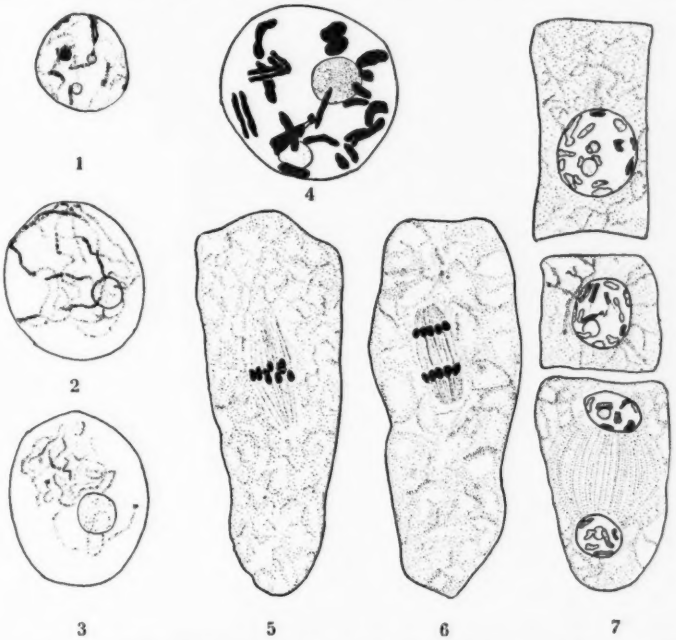
Observations

Monarda fistulosa

At first the macrospore mother nucleus is small, containing one to three nucleoli and chromosome-like bodies connected by fine threads. Figure 1 shows one of the latter bodies bearing a satellite attached to a nucleolus.

The macrospore mother cell and its nucleus rapidly enlarge. The leptonema threads are formed from the chromatic reticulum and

proceed to conjugate (fig. 2). In the synizetic contraction (fig. 3), the chromatic strands usually do not inclose the nucleolus but lie along one side and in contact with it. As only one nucleolus is present at synizesis, it seems likely that fusion of nucleoli has occurred in



FIGS. 1–7.—Macrosporogenesis in *Monarda fistulosa:* fig. 1, nucleus of archesporial cell; fig. 2, nucleus of macrospore mother cell, presynizetic stage; fig. 3, same, synizesis; fig. 4, diakinesis; fig. 5, lateral view of heterotypic equatorial plate; fig. 6, lateral view of heterotypic anaphase; fig. 7, two chalazal macrospores; micropylar cells not yet formed. ×1300.

those nuclei which previously contained more than one. As the chromosomes emerge from synizesis, their double nature is evident. After the shortening and thickening of the chromosomes, sixteen pairs can be counted in diakinesis (fig. 4). Size differences exist and the chromosomes may be grouped into eight larger and eight smaller pairs; in each group there is a gradation in size. Two large chromosome pairs bear satellites.

At the equatorial plate stage the chromosomes are smaller and more compact (fig. 5). The behavior of the chromosomes in metaphases and anaphases is regular (fig. 6). Satellite chromosomes can be identified in many of the nuclei of the macrospores (fig. 7).

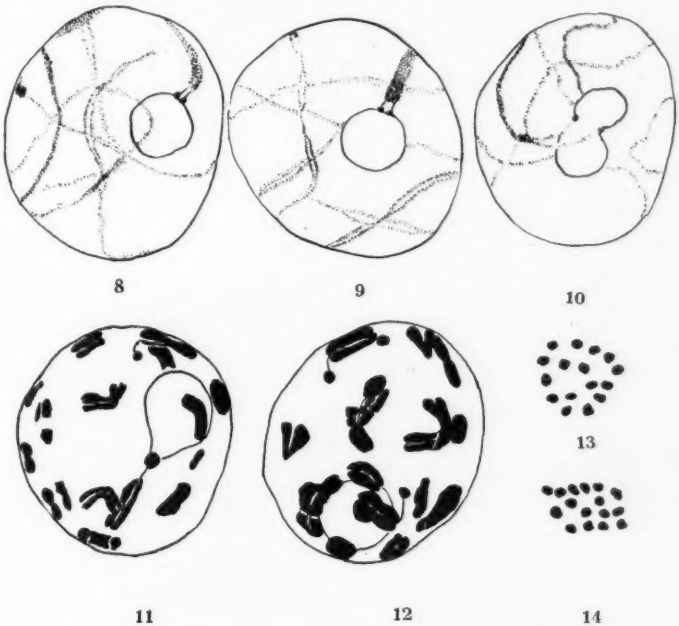
The nucleus of a young microspore mother cell has a chromatic reticulum and one, two, or three nucleoli. When there is only one nucleolus, it is large and probably the result of fusion.

The synizetic knot does not surround the nucleolus which, however, is always in contact with some of the strands. Occasionally a chromatic thread can be seen bearing a pair of satellites that are attached to the nucleolus, but generally the massing of the threads makes it impossible to distinguish such an attachment. When the threads of the synizetic knot become loosened, the loops of the spireme appear double. Small satellites and their fibers at the end of a chromosome can frequently be seen attached to the periphery of the nucleolus (figs. 8, 9). As the chromosomes are loosely paired, both satellites of the pair are distinguishable; they appear to be of about the same size. In some nuclei the nucleolus is constricted; in such a case satellites and their fibers partially encircle the nucleolus at the constricted region (fig. 10). In diakinesis sixteen chromosome pairs can be counted (figs. 11, 12). As in the macrospore mother nuclei, there are eight larger and eight smaller pairs, those in each group differing in size. Two pairs of large chromosomes bear relatively large satellites. One pair of satellite chromosomes is usually in contact with the nucleolus; occasionally this pair lies near the nucleolus but not in contact with it. On the other hand, the satellites of this chromosome pair are not always visible, perhaps having become appressed against the chromosomes. The chromosomes of this pair are most often attached to the peripheral portion of the nucleolus, and in some cases the nucleolus appears drawn out at the point of attachment (fig. 11). The other pair of satellite chromosomes apparently is never attached to the nucleolus.

The bipartite nature of the distal tip of a pair of satellite chromosomes is sometimes visible, but generally the two satellites of the pair lie so close together that they appear as one. In one instance one member of a pair of satellite chromosomes that was free from

the nucleolus has its satellite at one end while the other member has its satellite attached at the opposite end (fig. 12).

At diakinesis some nuclei contain two or three nucleoli which have



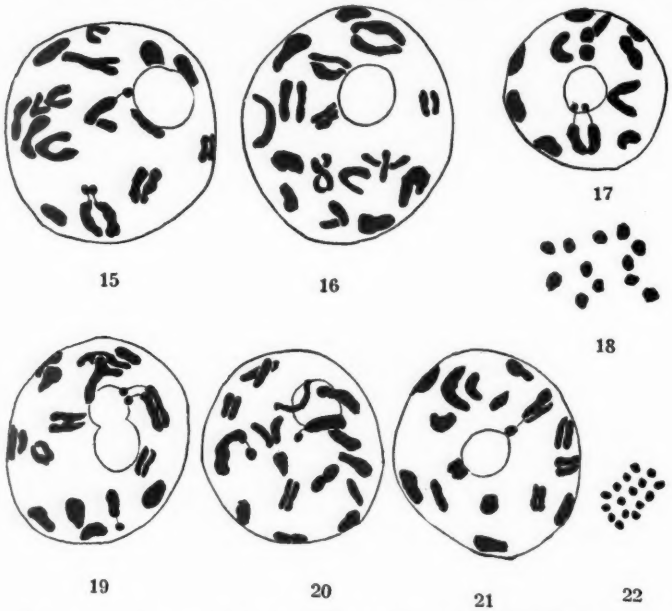
Figs. 8–14.—Meiotic divisions in *Monarda fistulosa:* Figs. 8, 9, open spireme stages showing satellite chromosome pair attached to nucleolus. Fig. 10, open spireme stage, satellite chromosome partially encircling bilobed nucleolus. Fig. 11, diakinesis; sixteen chromosome pairs, one pair of satellite chromosomes attached to nucleolus (which is distorted in shape) and one pair free from the nucleolus. Fig. 12, diakinesis; sixteen chromosome pairs, one pair attached to nucleolus and other pair bearing one satellite at one end, the other satellite at opposite end. Figs. 13, 14, polar views of homoeotypic equatorial plates each showing sixteen chromosomes. ×2830.

apparently arisen as a result of budding; other nucleoli at this stage are budding. Such budding has been observed only after synizesis.

Polar views of heterotypic equatorial plates show clearly sixteen pairs of chromosomes. Sixteen split chromosomes are visible in each sister nucleus in interkinesis. In some nuclei at this stage in which

the chromosomes are well distributed, the satellite chromosomes are visible and one pair is seen attached to the nucleolus.

In the homoeotypic division the chromosomes can be counted in



FIGS. 15–22.—Fig. 15, *Monarda didyma*, sixteen chromosome pairs; two pairs of satellite chromosomes, one of which is attached to nucleolus. Fig. 16, *M. didyma*, diakinesis, sixteen chromosome pairs. Fig. 17, *M. punctata*, twelve chromosome pairs, one pair of satellite chromosomes attached to nucleolus. Fig. 18, *M. punctata*, polar view of heterotypic equatorial plate showing twelve pairs of chromosomes. Figs. 19–21, *Nepeta cataria*, diakinesis; sixteen chromosome pairs, one pair of satellite chromosomes attached to nucleolus and one pair not attached. Fig. 22, *N. cataria*, polar view of homoeotypic equatorial plate showing sixteen chromosomes. ×2830.

polar views of the equatorial plate (figs. 13, 14). After their formation the four microspore nuclei pass into a resting stage. One, two, or three nucleoli are at first distinguishable, later only one.

Monarda didyma

Sixteen chromosome pairs are present in the nucleus of the microspore mother cell at diakinesis (figs. 15, 16). The nucleus at this

stage is of about the same size as that of *M. fistulosa*, and in this species also there are two pairs of satellite chromosomes (fig. 15). One pair is attached to the nucleolus; the other is free. Polar views of homoeotypic equatorial plates show sixteen chromosomes, the haploid number.

Monarda punctata

Twelve chromosome pairs appear at diakinesis (fig. 17). Only one pair of satellite chromosomes is present, the satellites being attached to the nucleolus. The nucleus is smaller than in the other two species of *Monarda*. Lateral and polar views of heterotypic metaphases show twelve chromosome pairs (fig. 18).

Nepeta cataria

The nucleoli just before synizesis appear bilobed and the lobes are of equal size. Since only one nucleolus is present at synizesis, the nucleoli fuse during the presynizetic stages. In *Monarda*, fusion of nucleoli was thought to occur, but was not observed. In *Nepeta*, however, fusion is apparently a slow process and observable in all nuclei.

In diakinesis, sixteen pairs of chromosomes can be counted (figs. 19–21). Two large chromosome pairs bear satellites; one pair is always attached to the nucleolus. At interkinesis, each of the sister nuclei contains sixteen chromosomes, one of which bears a satellite attached to the nucleolus. Sixteen chromosomes are visible also in polar views of the homoeotypic equatorial plates (fig. 22). The microspore mother nuclei of *N. cataria* are smaller than those of *M. fistulosa* and *M. didyma* although they contain the same number of chromosomes.

Summary

1. The haploid chromosome number in *Monarda fistulosa*, *M. didyma*, and *Nepeta cataria* is sixteen, in *M. punctata* twelve.

2. In *M. fistulosa* one pair of satellite chromosomes is commonly in contact with the nucleolus at synizesis, the open spireme stages, diakinesis, and interkinesis. A second pair of satellite chromosomes is free of the nucleolus in diakinesis and interkinesis.

3. Two pairs of satellite chromosomes are present in *M. didyma*

and *N. cataria*. One pair is attached to the nucleolus, the other pair free, at diakinesis and interkinesis.

4. Only one pair of satellite chromosomes was observed in the nuclei of *M. punctata* at diakinesis. It is attached to the nucleolus.

The writer wishes to express appreciation to Dr. C. E. ALLEN for valuable advice and criticism during the progress of the work.

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CYTOLOGICAL STUDIES IN THE RESEDACEAE

O. J. EIGSTI

(WITH THIRTEEN FIGURES)

Introduction

The phyletic relationship of species in the Resedaceae has already been established, utilizing floral and morphological features. The present work was undertaken to show how cytological information may be applied to the problems of phylogeny. Apparently no cytological work has been published dealing with the problem of relationship in species of *Reseda*. Accordingly a study was made through chromosome numbers and chromosome morphology, of the species formation and species relationship in this genus.

In the summaries by GAISER (1) on chromosome numbers no report of species in Resedaceae can be found. TISCHLER (6) lists four species, with a reference to a paper by OKSIJUK (4). The chromosome number summaries (table I) include those by OKSIJUK (5), as well as counts by the writer.

Material and method

The seeds of *Reseda* species were secured from botanical gardens in America and abroad, and the plants used for cytological material were grown at the University of Illinois and at the Carnegie Institution of Washington, Cold Spring Harbor, New York.

Chromosome counts were based on dividing pollen mother cells, and chromosome morphology was studied from dividing root tip cells. The aceto-carmine method was used for pollen mother cell studies. For root tip studies, various fixatives such as those of Allen, Navashin, Lewitsky, and Flemming were used. The material was imbedded in paraffin and sections were cut at approximately 7 μ. Most of the material was stained with iron-alum haematoxylin.

The counts in all cases were taken from pollen mother cells in the second meiotic metaphase. Chromosome studies in the root tips were made from polar views of the dividing nucleus.

Results

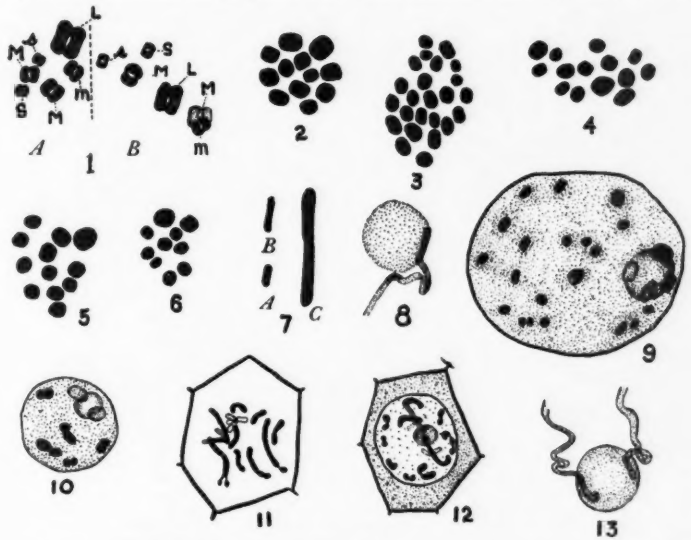
Figures 1 to 13 show the important features of chromosome morphology in species of the Resedaceae. The chromosomes are very small when compared with those of *Allium* (fig. 7*A*, *C*), although there

TABLE I

CHROMOSOME COUNTS IN THE RESEDACEAE

GENUS	n	2n	INVESTIGATOR
	Section Resedastrum Duby		
Reseda			
odorata L.	6	12	4; present paper
inodora Rchb.	6	12	5; present paper
phyteuma L.	6	12	5; present paper
media Lag.	6	.....	5
lanceolata Maire	12	24	Present paper
crystallina Webb	12	24	Present paper
lutea L.	24	48	Present paper; 5
diffusa Ball	24	.....	Present paper
stricta Pers.	24	.....	Present paper
	Section Leucoreseda DC.		
alba L.	10	20	4; present paper
	Section Luteola		
luteola L.	12	.....	Present paper; 5
luteola L.	13	.....	Present paper
	Section Glaucoreseda DC.		
glauca L.	14	.....	Present paper; 5
glauca L. (variations)	10	.....	Present paper
glauca L. (variations)	12	.....	Present paper
virgata L.	14	.....	Present paper
complicata Bory.	14	28	Present paper
complicata Bory. (variations)	15	.....	Present paper
Astrocarpus Neck.			
sesamoides J. Gay.	10	.....	5

is considerable size differentiation (figs. 1, 11, 12). One species studied (fig. 1), with six pairs of chromosomes, diploid number 12, reveals five classes of chromosomes which are as follows: one large pair (*L*), two medium large pairs (*M*), one medium sized pair (*m*), one



FIGS. 1.–13.*—Figs. 1–6, pollen mother cells of: 1, *Reseda odorata* (*L*, *M*, *m*, *S*, *s*, size classes of chromosomes in a species with 6 pairs); 2, *R. crystallina;* 3, *R. lutea;* 4, *R. glauca;* 5, *R. luteola;* 6, *R. alba.* Fig. 7: *A*, *B*, chromosomes of Resedaceae; *C*, of *Allium.* Fig. 8, *R. phyteuma*, meiotic prophase nucleolus and attached chromatin strand. Fig. 9, *R. crystallina*, nucleolus and two pairs of prochromosomes. Fig. 10, *R. odorata*, nucleolus and single pair of prochromosomes. Fig. 11, same, polar view of mitotic metaphase. Fig. 12, *R. alba*, prophase in root tip cell. Fig. 13, *R. lanceolata*, two chromatin strands attached to nucleolus in meiotic prophase.

* All figures drawn with Abbé camera lucida at table level; Zeiss 2 mm. apochromatic objective, N.A. 1.3, and compensating ocular no. 18 used.

medium small pair (*S*), and one small pair (*s*). Studies of the somatic chromosomes (figs. 11, 12) confirm the size differentiations, and additional features such as satellites and primary differentiation (fig. 11), secondary differentiation, and the chromosome-nucleolus association (figs. 8, 9, 10, 12, 13). In all the cases where somatic chromosomes were studied, spindle fiber attachments are median (fig. 11) and thus

produce equal bi-armed chromosomes. The satellites found in a twelve chromosome species occur on the largest pair and may be found dividing before the main body of the chromosome (fig. 11).

Prochromosomes are present in all the species of the Resedaceae studied. One, two, or three pairs of prochromosomes may be attached to the nucleolus during interphasic stages (figs. 9, 10, 12). Chromatin strands in meiotic prophase stages are in contact with the nucleolus (figs. 8, 13).

Chromosome counts 6, 12, and 24, which are haploid numbers, represent a euploid polyploid series in the section Resedastrum of the genus (figs. 1, 2, 3). The other sections in this genus, Glaucoreseda and Leucoreseda, have chromosome numbers of 14 and 10 respectively (figs. 4, 5).

Discussion

HELLWIG (2) and MUELLER (3) have treated the Resedaceae taxonomically. *Reseda*, which contains about 80 per cent of the total number of species in the family, is divided into four sections. These sections represent morphological, taxonomic, and cytogenetic groups of species. The representatives of each section analyzed cytologically give additional evidence for separation of species into the four groups.

A brief summary of these sections is as follows: Resedastrum, flowers 3 carpeled, stamens numerous (15–40), leaves entire or ternately divided; Luteola, flowers 3 carpeled, apocarpous, stamens yellow, leaves entire; Leucoreseda, flowers 4 carpeled, 5 or 6-merous, stamen number reduced (12–16), leaves pinnate or bipinnate; and Glaucoreseda, flowers 4 carpeled, 6-merous, stamen number (14–22), leaves entire, linear and grass-like in appearance.

The chromosome counts of a species in any given section of *Reseda* are characteristic and representative of the particular section to which they belong. For instance, Resedastrum has eight species with 6, 12, and 24 pairs of chromosomes, or a possible basic number of 6 which characterizes the species in this section. Leucoreseda is represented by *R. alba* with a chromosome complement of 10 pairs; Glaucoreseda with three species studied is characterized by a chromosome complement of 14 pairs. The fourth section Luteola, with

a single species, has a representative and characteristic chromosome complement of 12 pairs. In sections Glaucoreseda and Luteola, variations in the chromosome complement appear which can be explained by meiotic and mitotic abnormalities.

The grouping of species arranged by cytological studies according to chromosome numbers coincides with the grouping of species by their morphological features, and also coincides with grouping of species according to previous taxonomic studies. Cytologically, Resedastrum has species with 6, 12, and 24 pairs of chromosomes in their respective complements, and morphologically the species in this section have 3 carpeled ovaries and ternately divided leaves. On the other hand, Leucoreseda, with a representative species having 10 pairs of chromosomes, has a 4 carpeled ovary and pinnately divided leaves. A third and still different situation is in the section Glaucoreseda which has three species with 14 pairs of chromosomes. These species have 4 carpeled ovaries, and entire, linear, grass-like leaves. The fourth section, Luteola, is morphologically distinct from all other sections by the presence of a long entire leaf and a 3 carpeled apocarpous ovary, and a cytological entity with 12 pairs of small size, uniform chromosomes in the complement.

This coincidence of morphological and taxonomic features with cytological characters represented by chromosome numbers makes it possible to use the chromosome counts as additional information for a key to the species identification in the genus *Reseda*.

A euploid polyploid series is found in the section Resedastrum in the following species: *R. odorata*, *R. phyteuma*, *R. inodora*, have 6 pairs of chromosomes; *R. crystallina* and *R. lanceolata* have 12 pairs of chromosomes; and *R. lutea*, *R. stricta*, and *R. diffusa* have 24 pairs of chromosomes. Species formation in this group has undoubtedly occurred through interspecific hybridization and production of fertile plants by duplication of the hybrid complement, as postulated by WINGE (7) in a hypothesis suggested for the production of fertile hybrids.

The evidence for polyploidy in *Reseda* species is derived from the chromosome morphology, prochromosome-nucleolar association, and chromatin strand-nucleolar associations in meiotic prophase. In the first case, species with 6 pairs of chromosomes have five distinct

chromosome size classes and more critical morphological study gives a possible sixth size class. Those species with 12 pairs of chromosomes and 24 pairs of chromosomes in the complement do not have more than the six different size classes such as found in the complement of the 6 paired chromosome species. The species with a complement of 24 chromosomes have fundamentally 4 chromosomes of each of the six size classes, and species with a complement of 48 chromosomes have 8 chromosomes of each of the six respective size classes. Second, the species with 6 pairs of chromosomes show a single large pair of prochromosomes attached to the nucleolus during interphase stage (fig. 10), while those species with 12 pairs of chromosomes have two large pairs of prochromosomes attached to the nucleolus (fig. 9). In the 24 pair chromosome species, three pairs of prochromosomes have been observed attached to the nucleolus. Finally, species with 6 pairs of chromosomes show a single chromatin strand at meiotic prophase attached to the nucleolus (fig. 8), whereas species with 12 pairs of chromosomes have two chromatin strands attached to the nucleolus in meiotic prophase (fig. 13).

The presence of "capsella type" prochromosomes in all species studied suggests an affinity of the Resedaceae with the Cruciferae, in which prochromosomes are known to occur. This phylogenetic relationship proposed on the basis of prochromosomes confirms taxonomic and morphological studies made over a century ago which connected the Resedaceae with the Cruciferae.

Summary

1. Cytological studies of species of *Reseda* support taxonomic classifications made over a century ago.

2. Species with a multiple number of chromosomes are found in the section Resedastrum. These species have probably originated through duplication of the sets of chromosomes.

3. The basic number of the section Resedastrum is 6.

4. Chromosome numbers 10, 14, and 12 are found to be characteristic for species studied in sections Leucoreseda, Glaucoreseda, and Luteola respectively.

5. The chromosomes of *Reseda* are small when compared with those of *Allium*.

6. Satellites are present on the large chromosomes.

7. The spindle fiber attachments are median and thus produce bi-armed chromosomes which are characteristic for the Resedaceae thus far studied.

8. Five size classes of chromosomes can be distinguished in species with a haploid number of 6 chromosomes.

9. Prochromosomes of the "capsella type" are found in the somatic interphasic stages.

10. The prochromosome association with the nucleolus is not a random association but a specific one in which a particular pair is attached to the nucleolus.

The writer expresses his appreciation to Dr. J. T. BUCHHOLZ for suggesting this investigation and for his criticisms during the progress of the work.

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HISTOLOGICAL REACTIONS OF BEAN PLANTS TO INDOLEACETIC ACID

E. J. KRAUS,[1] NELLIE A. BROWN,[2] AND K. C. HAMNER[3]

(WITH THIRTY-THREE FIGURES)

Introduction

For many years problems of tissue differentiation and regeneration have received attention from workers in various fields. More recent activity in the study of some of the developmental phenomena of plants has resulted in the postulation of the existence of hormones which act as stimuli in determining the reactions of tissues as expressed in various environmental ranges. There are now available a rather wide variety of chemically pure compounds isolated from plants or synthesized chemically outside the plant, which are coming to be known commonly as growth substances or plant hormones. AVERY and BURKHOLDER (1) and WENT (13) have recently published reviews and summaries on this general subject.

Among the many substances being experimented with, indoleacetic acid has been shown by ZIMMERMAN and WILCOXON (14) and COOPER (4, 5) to stimulate root development in many plants, and by BROWN and GARDNER (3) to stimulate the production of overgrowths and tumors in the Red Kidney bean. These results, and a long cherished desire to know what histological changes take place in plants when they respond to a growth substance or hormone applied to them, have furnished the reason for beginning these studies.

The bean (*Phaseolus vulgaris*) has been selected as the first subject for detailed examination. In all the experiments here reported, involving some fifteen hundred plants, the variety Red Kidney was used. The plants were grown in pots containing garden soil and kept under average greenhouse conditions. After the seedlings had made

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sufficient growth so that the second internode of the primary axis, which extends just above the pair of heart-shaped leaves to the first compound leaf, had attained a length of 1 to 2 inches and the leaflets of the first compound leaf were beginning to spread out, the stem was cut off squarely about 1 mm. below the base of the petiole of the compound leaf (fig. 1). In some instances axillary shoots were used. All gave approximately the same results. After the shoot was cut

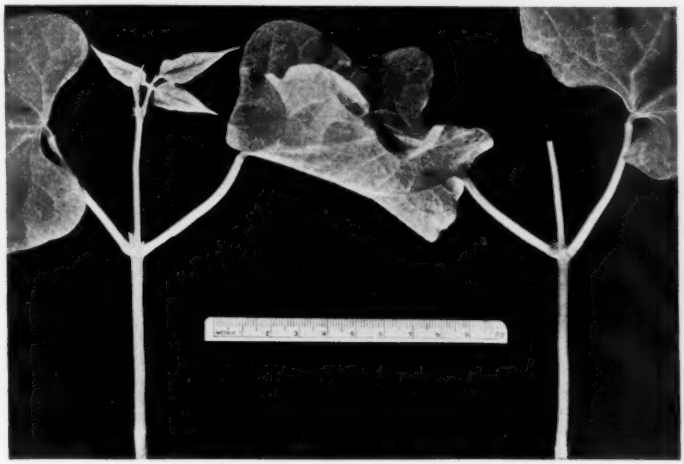

FIG. 1.—Seedlings of Red Kidney bean showing stage of development at beginning of experiments. At left untreated, at right decapitated and film of lanolin mixture applied on cut surface. Reduced.

back, a single application of lanolin containing indoleacetic acid in the proportion of 30 mg. per gram of lanolin was spread over the entire cut surface. Checks treated with pure lanolin and with no treatment other than cutting were also used. No particular attempt was made to regulate precisely the atmospheric moisture or temperature of the greenhouse, to manipulate the light supply, or to vary the soil conditions. What the average gardener would consider good growing conditions were maintained so far as possible. The temperature always ranged above 18° C. Little difference was noted in the character of growth or rate of response made by the plants, whether the experiments were made in the summer or autumn

months. Even so, repetition under a precisely controlled environment would be worth the effort.

Material for histological studies was collected at various intervals after decapitating and treating the stems. Navashin's solution was used throughout as a fixative. The butyl-alcohol paraffin method of imbedding was used and the sections made at 10 μ.

Gross responses to treatment

Following decapitation and the application of the lanolin mixture, very little gross response of the stem is noticeable within the first 18 hours; then a faint yellowing of the tissues adjacent to the mixture is evident. By the end of 24 hours the yellowing extends about 0.5 mm. down the stem and the ridges over the principal vascular bundles show a slight swelling just below the cut surface. Within 30 hours the yellowing has increased markedly, especially in the valleys between the ridges. Swelling of the topmost portion proceeds rapidly for at least 1 or 2 mm. down from the cut surface, and by the end of 48 hours the topmost portion of the stem is distinctly flared out. By the end of 72 hours the tip of the stem has attained a diameter nearly twice that of the stem 5 mm. below the cut surface, the whole upper portion is straw yellow, and the tissues in contact with the lanolin mixture are distinctly granular, resembling in appearance a new callus at the tip of a cutting of a root or shoot. The whole end of the stem continues to enlarge, as a rounded dome-shaped mass, and by the end of 110 to 120 hours the glistening tips of root primordia are evident about the whole periphery of the tumor adjacent to the line at which the original cut was made. Within 144 to 168 hours this crown of adventitious roots becomes more evident and some of them emerge beyond the surface of the tumor (figs. 2, 26). If the atmosphere is very moist these roots elongate rapidly and, if favorable conditions are maintained, may continue to grow indefinitely. If the atmosphere is somewhat dry, many of the roots may not emerge beyond the superficial tissues of the tumor, although they persist without further elongation for an indefinite period. If the atmosphere about them becomes saturated with moisture or if they are brought into contact with some moist medium, they may become active and elongate.

Even though the atmosphere remains dry, the tumor as a whole does not cease growth. Although the periphery of the tumor from 1 to 2 mm. from the edge, close to the base of the adventitious roots,

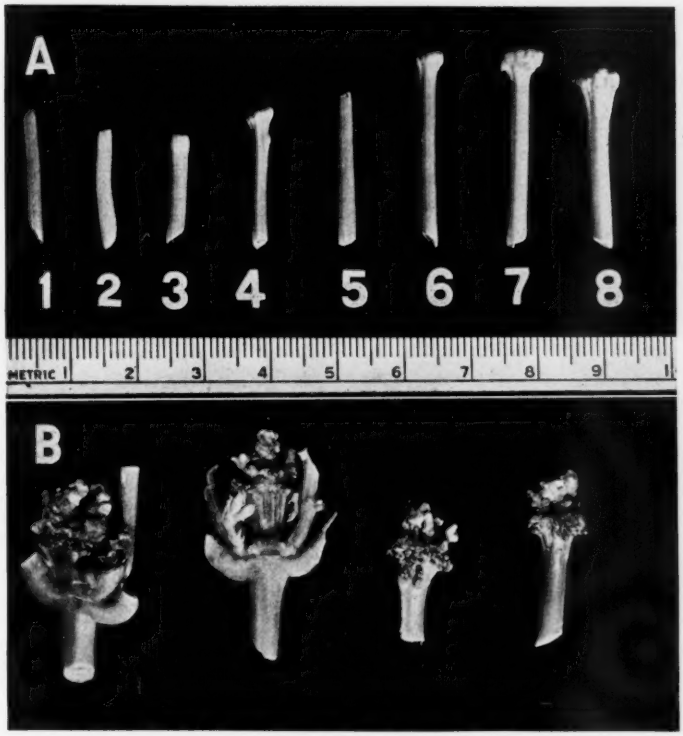

FIG. 2.—Stages in tumor development (time given in hours after treatment) *A: 1*, just after decapitation; *2*, 30 hours; *3*, 48 hours; *4*, 66 hours; *5*, 72 hours not treated; *6*, 72 hours treated; *7*, 144 hours; *8*, 168 hours. *B:* four apical tumors 28 days after decapitation and treatment of stem. Adventitious roots have not emerged, but prominent ridges and rows of small tumors over the vascular bundles are evident, as is also the central apical mass of proliferating tissue. Natural size.

may remain inactive, masses of tissue in the form of cones, balls, and other irregular and fantastic shapes grow upward and outward from the central portion, overtopping the crown of roots (fig. 2*B*). The tumors eventually may become 4 or 5 cm. in diameter. They

are gnarled and irregular in contour, greenish gray, with scurfy, gray patches scattered irregularly over their surfaces.

In general the stems which are cut back and treated with pure lanolin continue to grow slightly in diameter, remain dark green, and after a variable number of days develop a phellogen slightly below the plane of the cut (fig. 30). If neither lanolin nor the lanolin n ixture is applied to the cut surface, the tip of the stem may dry

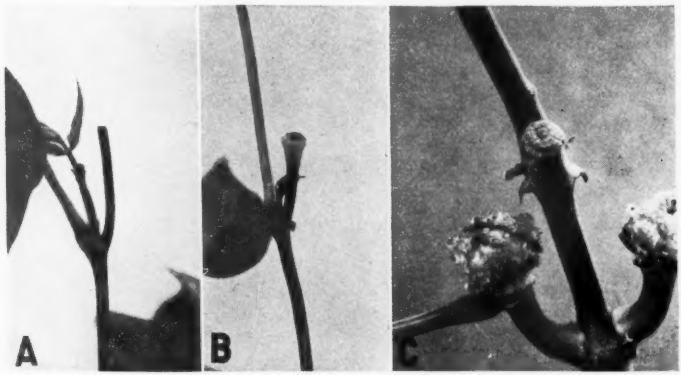

FIG. 3.—*A:* axillary branch 4 days after decapitation, no treatment. *B:* similar branch 4 days after treatment with lanolin mixture. Reduced. *C:* three branches 20 days after decapitation; upper center not treated but covered with callus, the other two treated at time of cutting with lanolin mixture. About natural size.

out, shrivel, and die back for a varying distance from the cut; or callus formation may begin near the periphery of the stem and continue until the entire end has been covered over by a dense, firm, somewhat wrinkled and superficially suberized mass of tissue (figs. 3*C*, 32). This callus is largely derived as a proliferation of the phloem (figs. 32, 33).

The tissues of the veins and petioles of the leaves, developing pods, and other parts of the plant readily proliferate and form tumors when the lanolin mixture is applied, whether or not the epidermal tissues are cut away or ruptured before such application is made (figs. 4, 5).

FIG. 4.—*A:* bean pods 16 days after being severed in half and cut surface treated with lanolin mixture. *B:* similar pods 28 days after being punctured with needle and treated at puncture with lanolin mixture. Slightly reduced.

Histological details

DOUTT (**6**) has described the normal anatomical details of the Black Valentine bean. The structure of the stem of the Red Kidney variety closely parallels her description. A transection of the second internode of the main axis taken at the level at which the plants were decapitated is shown in figure 6. It is obvious that most of the cells have not reached maturity, although the several main regions

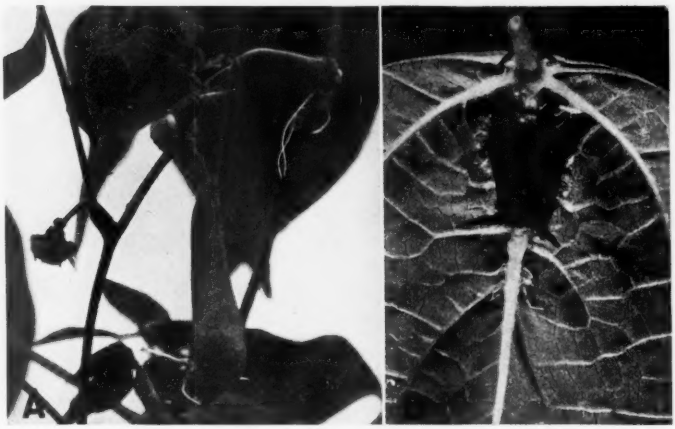

FIG. 5.—*A:* roots developed from end of bean pod 13 days after being severed and cut surface treated. Reduced. *B:* roots formed on midrib of bean leaf 18 days after severance of vein and local treatment with lanolin mixture. About natural size.

of the stem are well defined. The epidermal and cortical tissues, including the endodermis, have moderately dense cytoplasm and prominent nuclei. Many of the outer cortical cells have chloroplasts. The pericyclic cells over the primary phloem show the beginning of thickening to form fibers, but still have dense cytoplasmic contents and apparently nuclei which have not disintegrated. All the cells of the primary phloem are embryonic except perhaps the sieve tubes. The cambium is active; the zone of undifferentiated tissue is not more than four or five cells wide. The cells composing the medullary rays as well as those of the pith have dense contents but show no marked meristematic activity.

Eighteen hours after the application of the indoleacetic-lanolin mixture some definite changes are observable. The cells of the first one or two layers just below the treated surface are frequently killed, but beneath these the following changes are observable. The chloroplasts in the peripheral cells of the cortex have disappeared. The cortical cells have undergone marked enlargement; their cytoplasmic content appears much denser; and a few cells, especially those adjacent to the endodermis, show tangential divisions. The endodermal cells have enlarged markedly, especially in the radial dimension; their content is denser and starch grains are no longer readily demonstrable with the iodin test. The pericyclic cells have also increased in size, but their walls show very slight thickening. The parenchymatous cells of the primary phloem appear meristematic, as do those of the secondary phloem; the zone of undifferentiated cells adjacent to the cambium is much wider, owing to an increased number of cells and the larger size of many of them.

Subsequent developments, beginning with material which has been treated for 30 hours, are indicated in the photomicrographs. In some of the experiments specimens were taken at 6-hour intervals from the beginning up to a total duration of 168 hours. Only those examples which show significant progressive developmental changes are presented here. Not every stem used in each experiment responded at exactly the same rate, the more highly vegetative plants seemingly advancing more rapidly than those less vegetative; but in general any particular lot of plants subjected to the same type of treatment showed a high degree of uniformity. There were some marked differences in response of the plant as a whole and of the tissues in the vicinity of the application of the lanolin mixture, depending upon whether the epidermis was or was not injured, whether the applications were made on one side of the stem or completely encircled it, whether the application was in a thick or a thin layer, and other variations. We shall endeavor to present at a future time the details of these varied experiments, the histological pattern of tumors older than 168 hours and of the plants on which they are borne. The present discussion deals mainly with material not older than 168 hours.

One of the most obvious results of the application of the lanolin

mixture to the stem tissues of the bean is the marked stimulation to meristematic activity of some of the tissues and not others. This is first characterized by increased nuclear division. Some cells of the endodermis may have as many as six nuclei before any visible walls are formed, while in some of the larger cells of the pith as many as twelve or even more nuclei may be found.

In none of our experiments has the epidermis shown marked increase in meristematic activity. The cells may enlarge more rapidly than those of untreated stems. There is a slight increase in the number of radial divisions, but the cells do not divide tangentially. When the concentration of 30 mg. of indoleacetic acid to 1 gram of lanolin is used, the epidermal cells in immediate contact with the mixture are likely to be killed, but otherwise they persist for an indefinite period unless the expansion of tissues within ruptures the surface, or the cortical cells beneath them die.

The parenchymatous cells of the cortex show varying degrees of response. Those next to the epidermis enlarge, often very greatly, but may show no change other than an increasing density of the cytoplasm and the disappearance of the chloroplasts and any included starch grains. Those near the endodermis generally become highly meristematic, their nuclei undergoing rapid divisions, often with subsequent wall formation delayed, so that the cells are multinucleate. After 110 to 120 hours meristematic activity is greatly decreased near the cut surface; the outermost cells mature, die, and become disrupted by the expanding roots and other tissues formed centripetally to them. The cells which are 3 or 4 mm. distant from the cut surface are not so highly meristematic at any time as are those near it, and they frequently do little more than enlarge. They apparently remain alive for a considerable period, although as time goes on the chlorophyll disappears from them at distances of more than 5 cm. from the developing apical tumor.

Of all the cortical tissues, the endodermis is most responsive. Within 24 hours the cells near the treated cut surface enlarge greatly, starch disappears from them, their cytoplasmic contents become dense, and soon thereafter rapid nuclear divisions begin. These original cells generally elongate markedly in a radial direction. Sub-

sequently tangential divisions occur, and the original single layered endodermis becomes a wide meristematic band consisting of cells which are often indistinguishable from those of the adjacent cortical parenchyma. In the vicinity of the apical tumor this band of cells remains active for a long period and some of the cells composing it may mature as tracheids with large pits, others as parenchymatous cells with large vacuoles, while many form sheath-like coverings over the developing roots.

The behavior of the endodermal cells at distances of 2 mm. to as much as 3 cm. from the apical tumor, and hence well away from any direct contact with the indoleacetic acid except as it finally diffuses to them through other tissues, is of special importance. The reactivity and multiplication of longitudinal columns of these cells so closely parallel the tumor strands of crowngall that they may well be considered as being analogous to and perhaps homologous with them. Similar strands are developed in other tissues also, as described later. As indicated in figure 17, within 72 hours endodermal cells distant at least 1 cm. from the treated surface show marked radial enlargement and frequently tangential divisions as well. They do not, however, become so highly meristematic as those close to the developing apical tumor; but the derivatives undergo division in all planes, and form, especially over the primary phloem of the vascular bundles, groups of dividing cells concentrically arranged. The cells at the center of such a group develop into xylem elements; those nearer the periphery continue division and differentiation, so that in any given section it is possible to see at the end of 168 hours vascular strands with a central mass of xylem surrounded by dividing cells and phloem elements, the whole having been derived from cells of the original endodermal layer. At this time too, cells of the endodermis as far away from the cut surface as 3 cm. show the same type of activity as did those nearer it at a shorter time interval. Thus it is possible to observe within a 4 cm. length of stem all stages in development of these endodermal vascular strands, beginning with the first regions of activity at the lower level to large conspicuous strands with xylem elements at the center near the apical tumor (figs. 22, 23). After some days there

may be so great an enlargement of these and other strands developed in other tissues that the cortex is ruptured and small corrugated tumors appear along the stem.

Some specimens show root primordia originating in the outer layers of such active endodermal cells, accompanied by the differentiation of vascular structures through the intervening tissues back to the xylem of the original bundles in the stem. These are no doubt the beginnings of roots which later emerge from the stem at varying distances below the apical tumor.

The cells of the pericycle which would normally mature as fiber cells do not appear to become markedly meristematic in response to applications of the lanolin mixture. If they are relatively embryonic and lie adjacent to the treated surface they do enlarge and may undergo several divisions, but they do not elongate greatly nor do their walls become much thickened (fig. 18). They are most frequently crushed or pushed from their original position by the tissues located centripetally to them. Two or 3 mm. distant from the cut surface the walls of the pericyclic cells continue to thicken and the fibers mature much as they do in an untreated stem. The cells over the ray remain thin walled and undergo a few divisions, but often are not distinguishable as a tissue from the exceedingly active endodermal cells lying outside them or those of the ray nearest them.

The primary phloem is highly sensitive in its response to the stimulus of the indoleacetic acid, and near the surface of application the parenchymatous cells of this tissue proliferate abundantly and frequently elongate radially. The new cells thus formed may for a time be multinucleate, but later walls form in all directions, especially in the tangential plane. Eventually the cells enlarge and many of them mature as tracheids with large simple pits. Others remain parenchymatous and ultimately die, or become ruptured and torn by the expansion of other tissues near them. At greater distances from the tip the primary phloem cells undergo the same types of change and development as do those of the endodermis already detailed. Thus after 168 hours, strands of actively dividing cells located within the area of the primary phloem extend 2 or 3 cm. down the stem. Later these develop xylem elements at their

centers and may extend for much longer distances. Finally radial expansion and development result in masses of cells which rupture the tissues exterior to them and protrude as tumors and root initials of varying size in more or less parallel or longitudinal rows along the stem and downward from the large apical tumor.

The parenchymatous cells of the secondary phloem are as sensitive as are those of the primary, and are among the first tissues to show a decided response after the application of the lanolin mixture. Adjacent to the treated surface, meristematic activity of these cells is very great. The cells enlarge in all directions, particularly radially, thus pushing outward all tissues exterior to them and forcing the sieve-tube companion-cell groups wide apart and in all directions. This proliferated phloem tissue forms a large part of the developing apical tumor in its early stages and enters into the lateral portions of the adventitious roots which are eventually formed at the top of the stem. It is doubtful whether they contribute to the apical meristem of such roots when their histogens are eventually differentiated. At increasing distances down the stem away from the treated surface the parenchymatous cells are decreasingly active, and show far less response in such regions than do the cells of the endodermis, primary phloem, or the pith.

With the exception of the endodermis, the cambium is probably the most sensitive in its response to this type of stimulation. Its cells proliferate rapidly, but differentiation of the derivatives is much delayed. Thus a very wide band of highly meristematic cells comes to exist between those elements of the secondary xylem and phloem which were differentiated before the lanolin mixture was applied. Later large pitted tracheids mature more or less at random throughout this tissue and some of the cells near the phloem mature as phloem elements. The response of the cambial cells may be manifested several millimeters below the surface of application, but not at distances so great as in the case of the endodermis or the phloem, especially the primary phloem.

In point of time the cells of the pith are relatively slow to respond, but when meristematic activity is initiated the cells proliferate rapidly and their activity continues over a longer period of time than does that of the cortex (excepting the endodermis) or of the second-

ary phloem. Activity is first manifested by those cells which lie adjacent to the metaxylem vessels and the elements of the protoxylem. At first wall formation follows nuclear division very closely, but later many of the cells may be multinucleate, wall formation taking place subsequently. Activity of these parenchymatous cells often forces the elements of the xylem apart and their linear arrangement becomes greatly confused. Activity is begun somewhat later by the larger cells of the pith, but eventually practically all the cells differentiated before the lanolin mixture was applied become highly active. Throughout the mass of resultant tissue there are enlarged parenchyma cells, strands, zones, and islands of meristematic cells, tracheids, and phloem elements in every possible relationship. It is this conglomeration of tissues which continues to develop over a very long period of time and eventually makes up a large part of the apical tumor which assumes varied and grotesque shapes, as mentioned previously.

The cells of the ray parenchyma which lie between the points of the xylem respond almost precisely as do those of the pith, except that larger numbers of them mature more rapidly as pitted tracheids. Many of the cells remain meristematic for a long time and some of the derivatives of these also mature as tracheids and as clusters of sieve tubes. The cells of the ray which lie on a line with those of the phloem behave much as do those of the phloem parenchyma. Meristematic activity is very great, and as such activity continues and the derived cells nearer the center of the stem enlarge, a dense meristematic mass, flanked at either side by the highly active phloem parenchyma, is pushed outward toward the periphery of the stem. In some specimens this mass occupies a comparatively narrow wedge between the phloem groups; in others it is much wider. Often it is difficult to define exactly, but its limits can generally be decided from the patches of sieve tubes which lie at either side. In stages of development between the ages of 66 and 80 hours, masses of cells with denser content can be seen just within the band of endodermal cells; and after 80 hours the outlines of adventitious roots are readily discernible, made up partially of ray cells and partially of cells of the phloem.

The development of a circle of adventitious roots within a dis-

tance of 1 or 2 mm. from the cut surface of the stem is one of the most striking characteristics of the bean stem when such surface is treated with indoleacetic acid in lanolin. The general histological responses of the several tissues have just been described. In the genesis of the roots several tissues are involved. It seems doubtful whether the pericycle plays much part. Over the rays there are two to four thin walled cells which undergo some divisions, but such divisions are not extensive either radially or tangentially. The cells of the ray immediately inside these, however, proliferate considerably. So rapid and so extensive are these divisions, that if histogens for the adventitious roots are developed by the ray cells in the early stages of tumor formation they are not clearly indicated. In fact, such a histogen is difficult to distinguish even in stages 110 hours old, although groups of more highly active cells occupying the position of the ray between two adjacent masses of phloem and even within the phloem areas are readily discernible in 48 hours (fig. 8). In stages of development older than 80 hours the outlines of the roots become well defined, owing to the enlargement and increasing vacuolization of the cells near the center of the phloem masses of each of the vascular bundles, over the xylem, and beneath the pericycle. The lateral cells of two adjacent phloem groups remain meristematic for a considerable period and serve to make up the bulk of the outer portion of any given root. The central core of each root is derived largely from ray cells, and its apical histogen seems to be derived from the same source. At least when it is possible to be certain of the apical histogen, the cells which compose it lie nearer the center of the stem than do those of the proliferated endodermis, and probably also those of the pericycle, although this is much more difficult to determine. Following this general blocking out of the root, xylem elements are differentiated near the base and periphery of the inner core of cells; many mature as large pitted tracheids abutting the tracheids already differentiated from those ray cells which previously lay between the xylem vessels of two adjacent bundles. Those nearer the center of the core elongate greatly and differentiate mainly as tracheids, although others appear as tracheal segments. Phloem elements mature in the outer portions, especially in contact with the sieve tubes and other cells

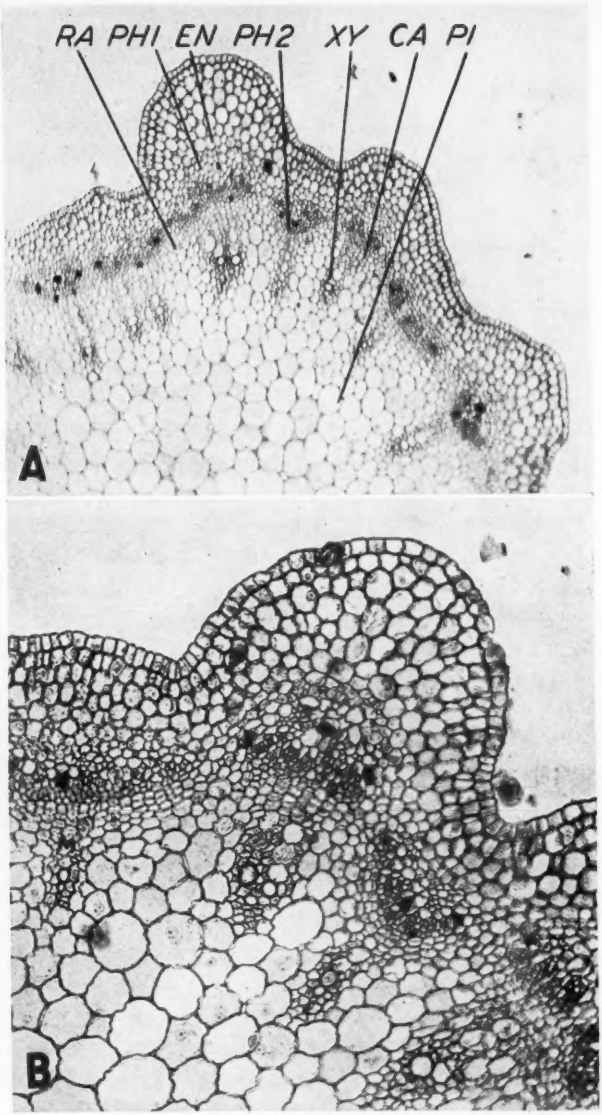

FIG. 6.—*A:* transection of top of second internode of bean at time of initial treatments, showing various tissues and stage of development (*ra*, ray; ph_1, primary phloem; *en*, endodermis; ph_2, secondary phloem; *xy*, xylem, *ca*, cambium; *pi*, pith). *B:* sector of same enlarged; pericyclic fibers over the smaller bundles are more nearly mature than those over the larger.

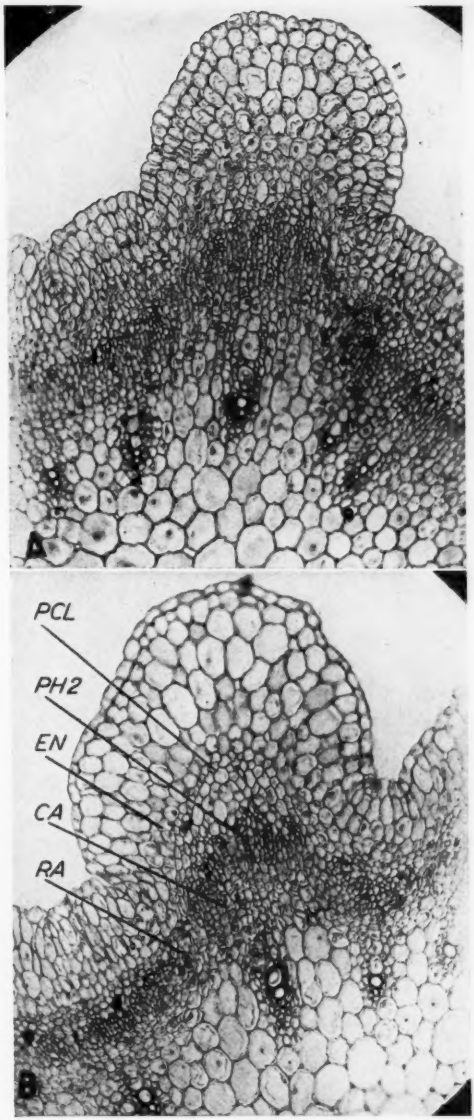

FIG. 7.—Thirty hours after treatment. *A:* section about 150 μ below treated surface. Pericyclic cells are embryonic, primary and secondary phloem parenchyma in stems show increase in meristematic activity, derivatives from the cambium remain meristematic and continue division, ray parenchyma is active, cytoplasm of the pith cells is dense. *B:* about 1.5 mm. farther down the stem. Pericyclic cells show increased maturity, other tissues about as in *A*.

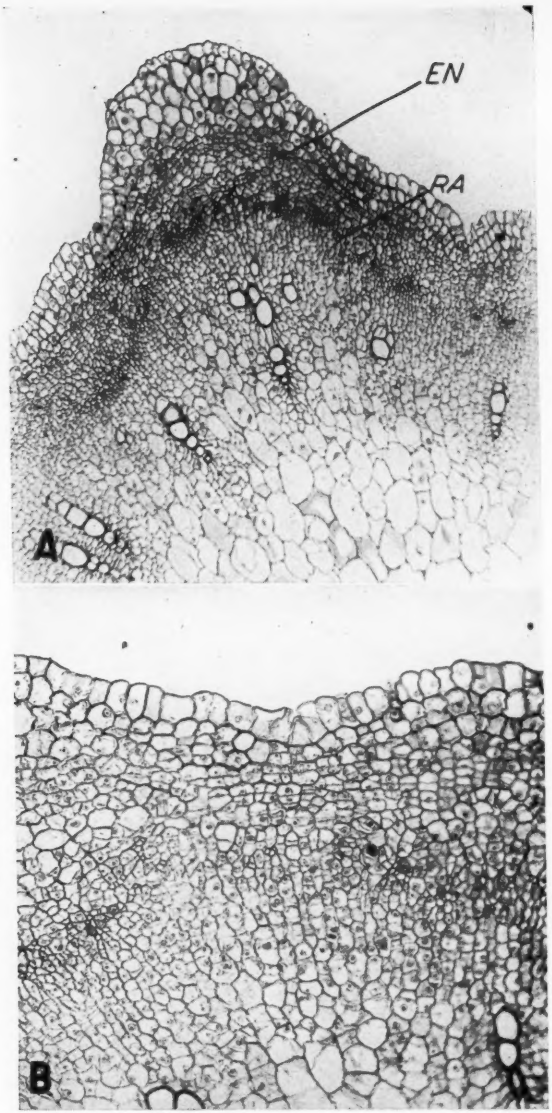

FIG. 8.—Forty-eight hours after treatment. *A:* section about 600 μ below treated surface. There is marked increase of meristematic activity of all tissues except the pericycle and epidermis. Some xylem vessels are pushed apart by active ray and xylem parenchyma cells. Pith adjacent to protoxylem and metaxylem is meristematic. There is slight differentiation of secondary tissues from the cambium. *B:* same enlarged, showing tangential division of cortical parenchyma and endodermis, also active ray cells.

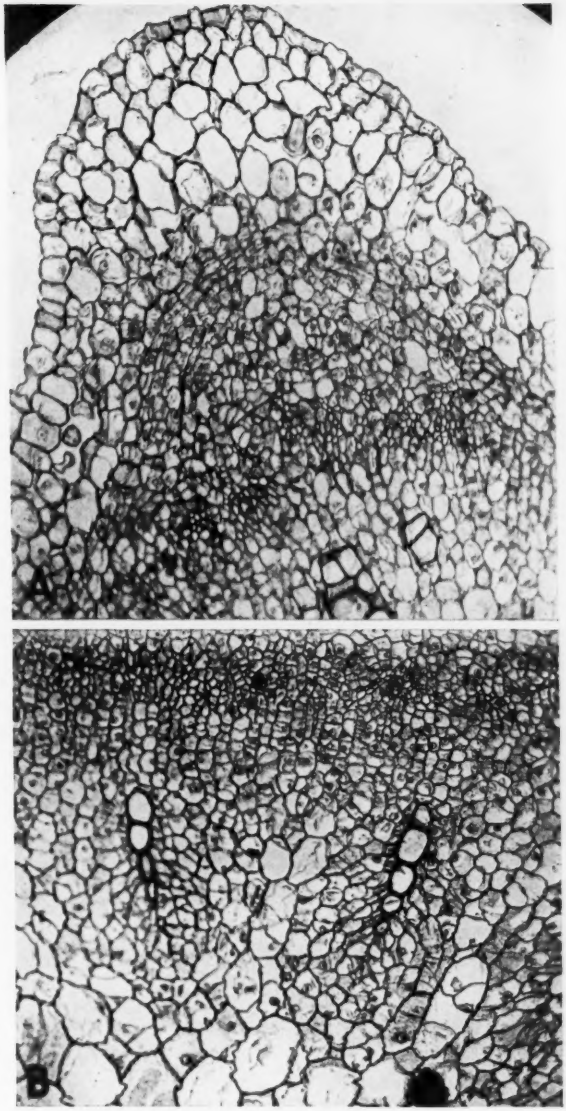

FIG. 9.—*A:* same stem as fig. 8; section about 1.5 mm. below fig. 8. All tissues except the endodermis are less active. Secondary elements from the cambium are fewer and more nearly mature. *B:* same as fig. 8 *B*, showing activity of pith and ray cells.

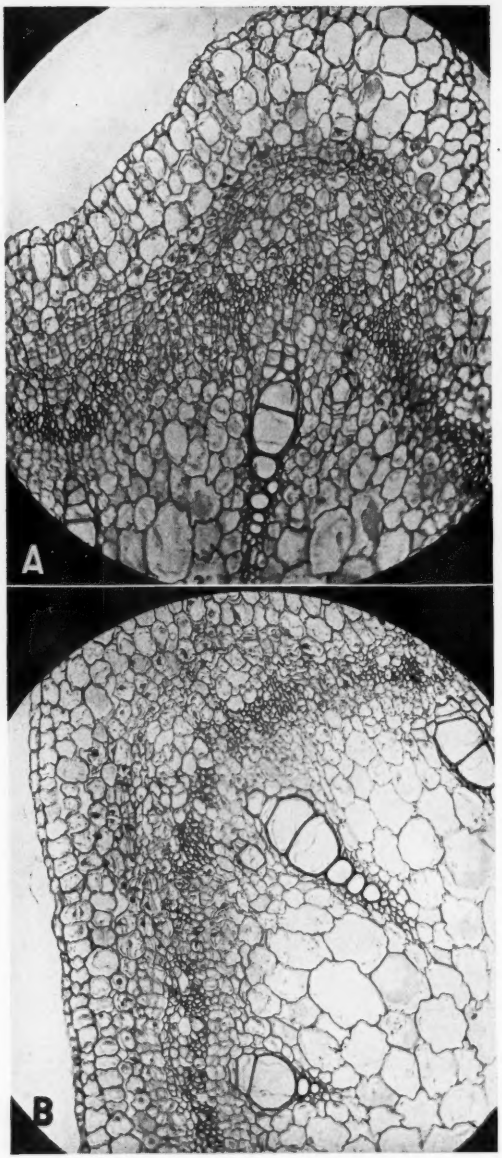

FIG. 10.—Same stem as figs. 8 and 9. *A:* section about 2 mm. below treated surface. Endodermis and primary phloem parenchyma are meristematic, the other tissues much more nearly mature. Cambial zone is narrow with few meristematic secondary derivatives. *B:* section 4 mm. below treated surface. With the exception of the cambium and endodermis, all tissues are nearing maturity. Endodermal cells, especially those over the smaller bundles, have undergone several divisions in tangential plane.

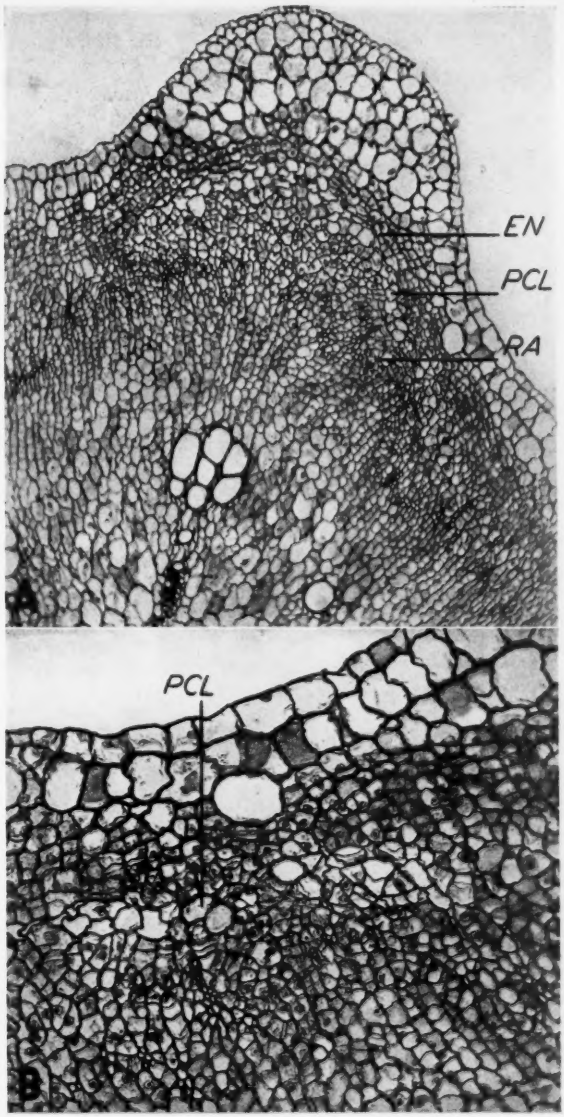

FIG. 11.—Sixty-six hours after treatment. *A:* section about 1 mm. below treated surface. Increased activity of all tissues except the pericycle as compared with 48-hour material. Pericyclic cells are comparatively much larger and their walls thicker; ray cells have proliferated considerably and the whole mass has expanded radially, paralleling the expanding phloem regions and derivatives of the cambial zone. *B:* enlarged view of ray in *A*. Pericyclic cells over it, on a line with those maturing over the bundles, show slight activity although the ray cells are much more active.

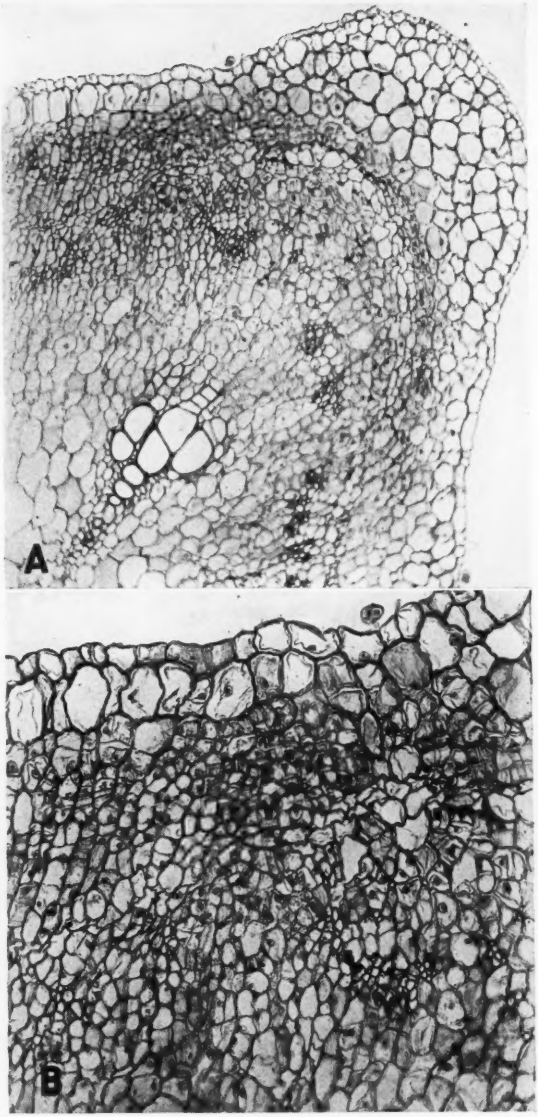

FIG. 12.—Same stem as fig. 11. *A:* section 5 mm. below treated surface. Less activity than in fig. 11, but because of longer time interval after treatment the tissues show greater meristematic activity than do those in fig. 10 *B*. *B:* ray and adjacent tissues enlarged; pericyclic cells over the bundles approaching maturity; endodermal derivatives and inner cortical parenchyma very active.

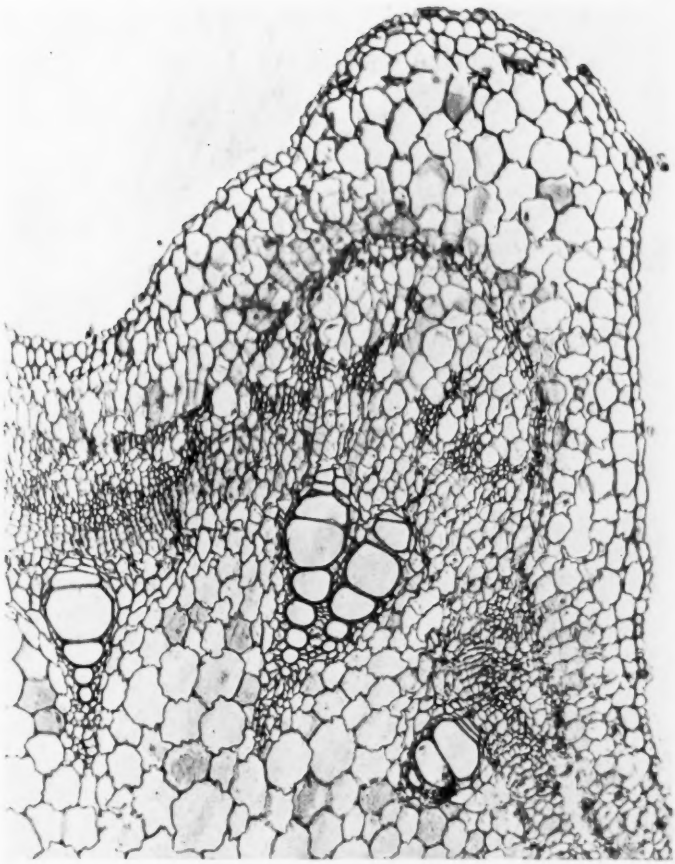

FIG. 13.—Same stem as fig. 11, section 9 mm. below treated surface. Endodermis, primary phloem of larger bundles, and pith near the protoxylem and metaxylem show some response; other tissues except the cambium nearing maturity. Rays show virtually no response. Slightly below this level no obvious response to the treatment (*cf.* fig. 17).

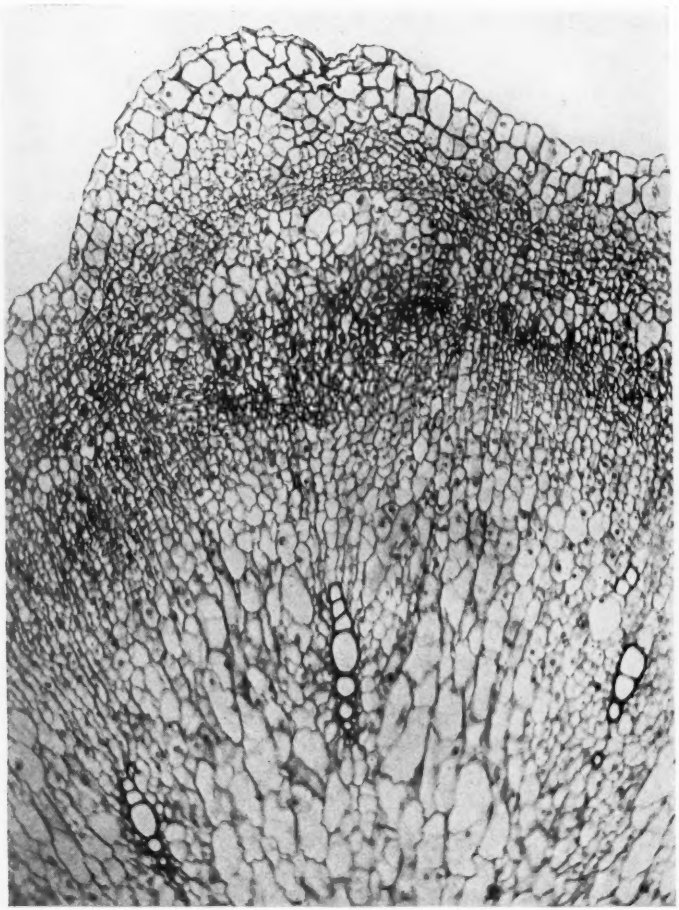

FIG. 14.—Seventy-two hours after treatment, section about 1 mm. below treated surface. Pericyclic cells much enlarged and nearing maturity although walls have not thickened greatly. Endodermal derivatives constitute a wide meristematic zone. Phloem parenchyma and derivatives from the cambium remain meristematic. Ray cells just within the pericycle constitute a meristematic mass distinguishable with some difficulty from the flanking phloem cells. The cells nearer the xylem vessels and between the bundles are meristematic and have elongated markedly in radial direction, thus pushing the outer cells of the ray toward the periphery of the stem.

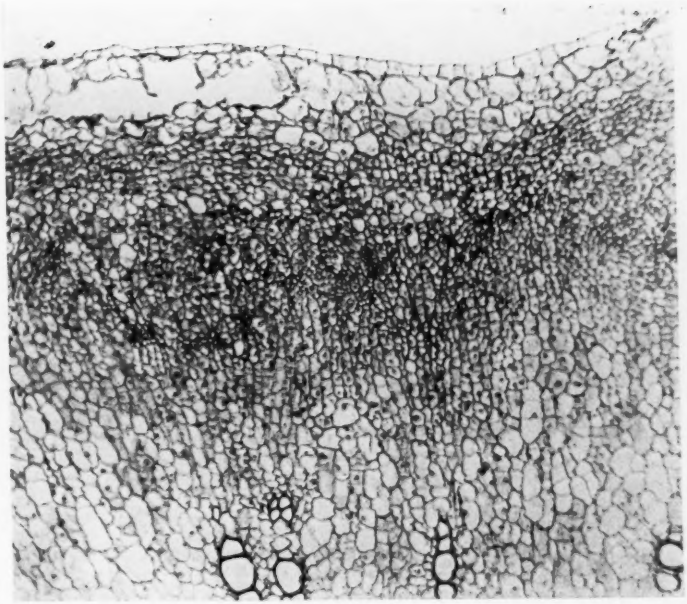

FIG. 15.—Same as fig. 14. Outer cortical cells disrupted by expansion of tissues within. The section lies in plane in which adventitious roots are formed. The outlines of such a root are shown in ray near center of illustration. At this stage it is very difficult to distinguish definite histogens for the new roots, if such histogens are present.

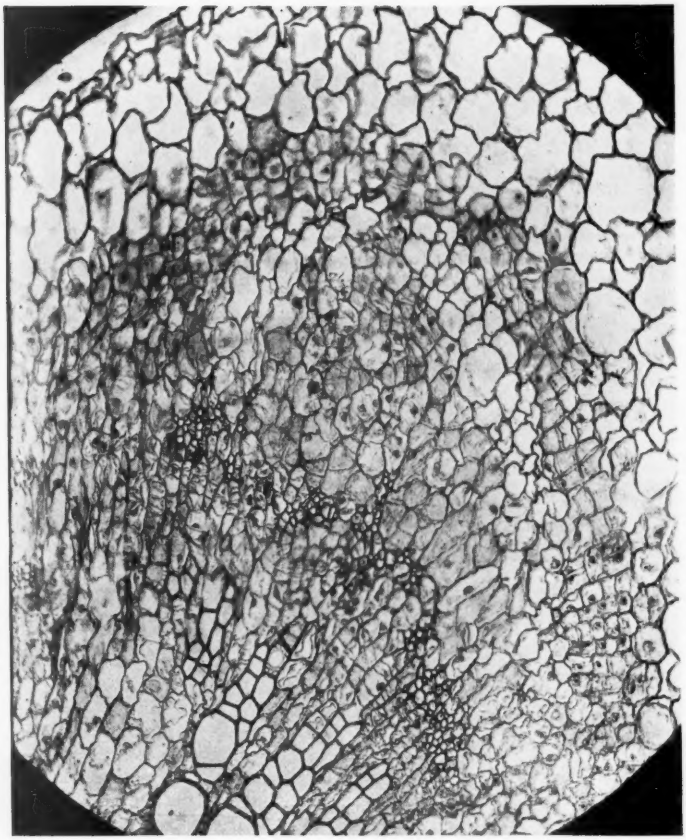

FIG. 16.—Same stem as fig. 14, section about 2.5 mm. below treated surface. Cambial derivatives have matured close to the cambium; pericyclic cells nearly mature; endodermis proliferated. Within the region of primary phloem there is great meristematic activity and near the center is the beginning of a vascular strand. Such a strand at a somewhat more advanced stage is shown in fig. 21. Such strands eventually extend continuously for distances of several centimeters in the stem, the more nearly mature portions at the top and the younger progressively down the stem.

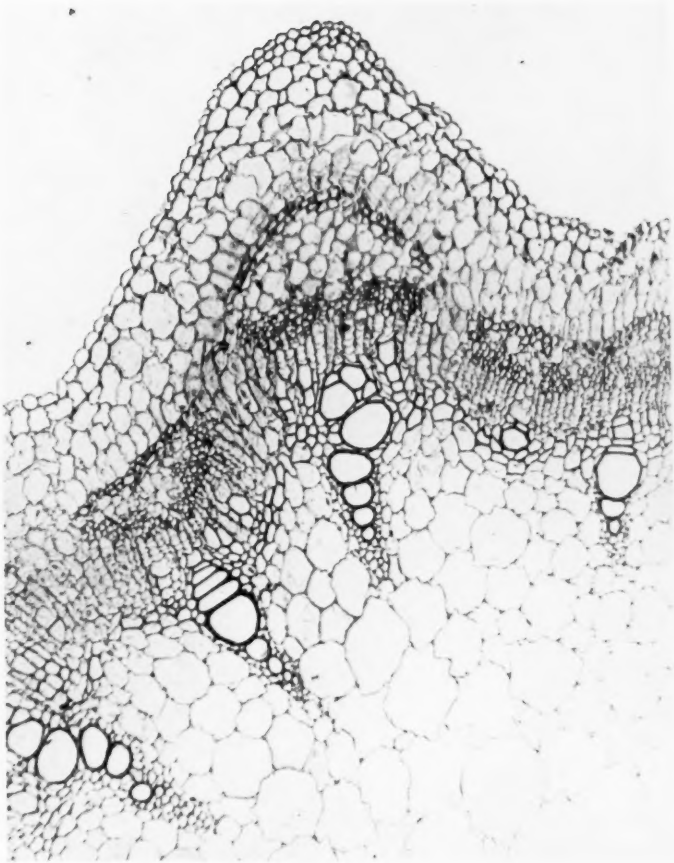

FIG. 17.—Same stem as figs. 14 and 16, section 1.2 cm. below treated surface (*cf.* fig. 13). Response is shown by cells farther away from surface of application and there are more tangential divisions of the endodermal cells. Sections of this stem taken 1.7 cm. from the treated surface showed the endodermal cells greatly elongated in a radial direction and tangential divisions of some of them. Some ray cells have matured as tracheids. The latter are abundant in the secondary xylem. The fibers over the primary phloem are not yet fully mature but show no divisions.

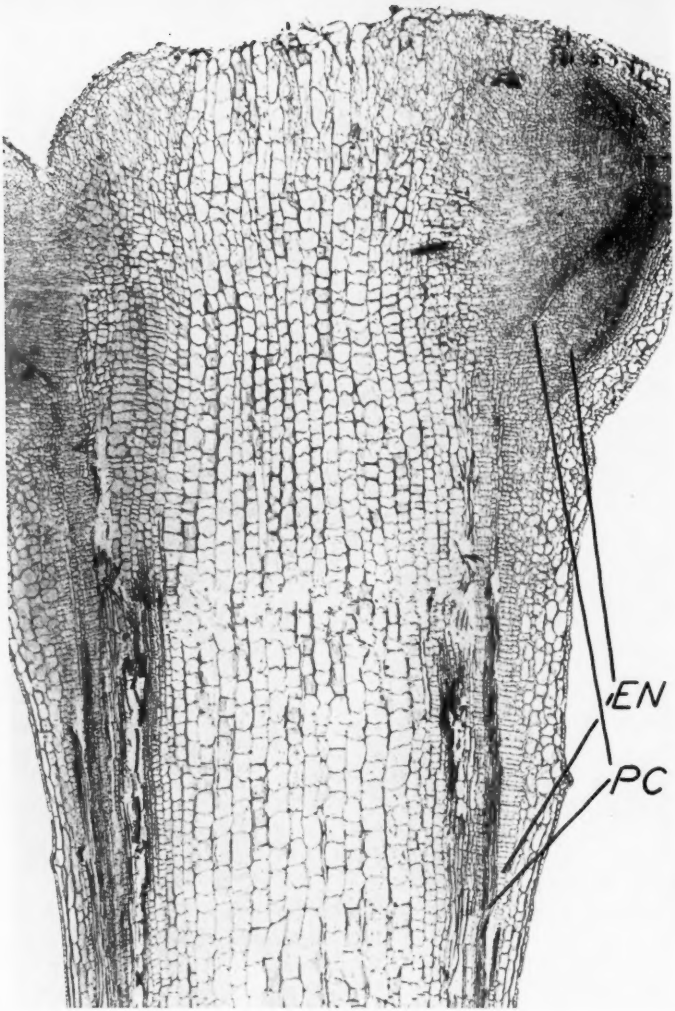

FIG. 18.—Ninety hours after treatment, median longitudinal section. Lower portion of adventitious root, nearer pith, is through a ray; distal portion is not strictly median. Pericycle cells (*pc*) have matured as fibers in lower portions of stem, but in upper portions nearer the surface of application they have divided and enlarged as parenchymatous elements. The tip of the root lies just beneath this layer. Endodermal cells (*en*) have responded to stimulation for a long distance down the stem. Older stages would show such response at even greater distances. Proliferated phloem extends along margins of the adventitious root whose central portion is made up largely of proliferated ray cells. The pith is active, particularly near the top. Cortical parenchyma is slightly active.

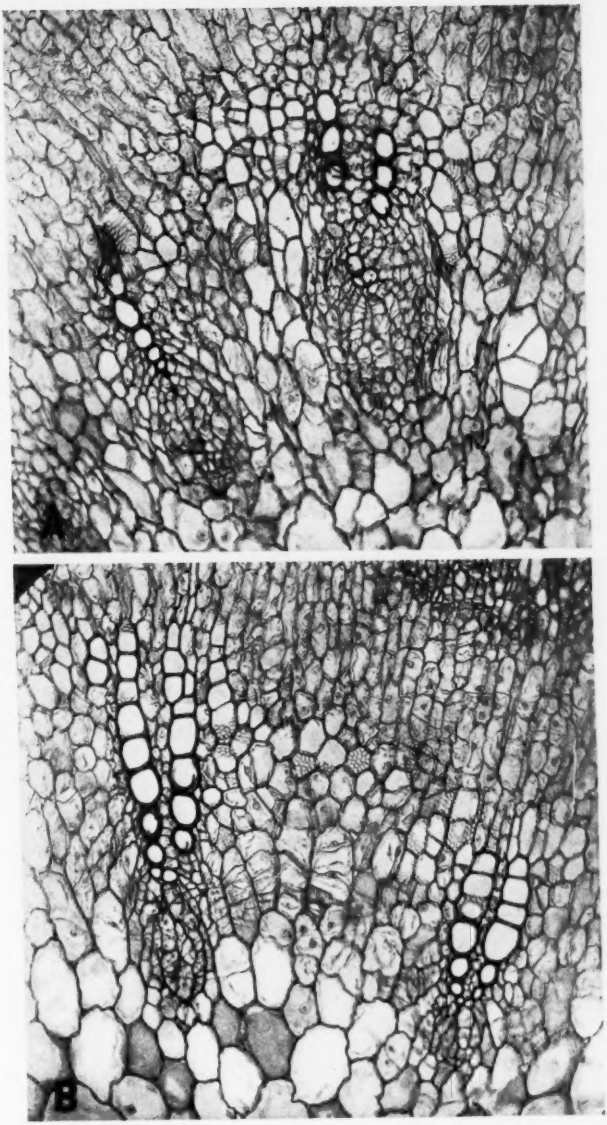

FIG. 19.—Eighty hours after treatment. *A:* section 1.3 mm. from treated surface. *B:* 1.6 mm. distant from surface. In both, the pith, ray, and xylem parenchyma cells are active and many of the cells have matured as large pitted tracheids across the rays and adjacent to the xylem.

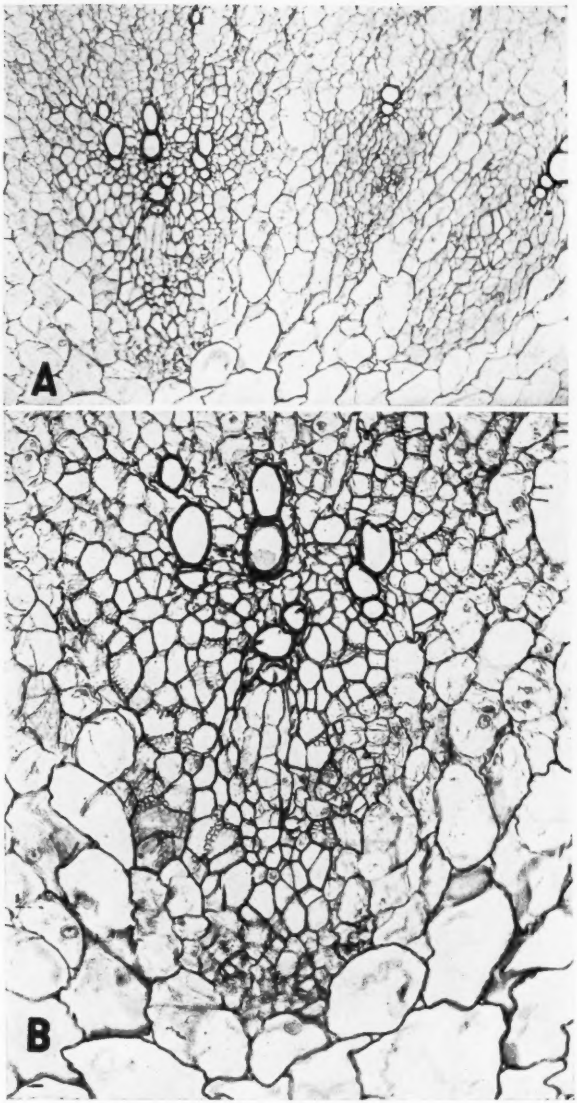

FIG. 20.—One hundred ten hours after treatment, section 1.4 mm. below treated surface. *B:* enlarged view of region at left in *A*. Active ray, xylem parenchyma, and pith cells. Some derivatives have matured as pitted tracheids; others as phloem elements; many still highly meristematic.

FIG. 21.—One hundred ten hours after treatment, section 5 mm. below treated surface. Tissues other than endodermis and primary phloem show little response to treatment. In the primary phloem a vascular strand has differentiated. At center of strand are large pitted tracheids surrounded by meristematic cells some of which would later differentiate as tracheids, and others continue to divide. At upper left of this bundle sieve tubes are differentiated just within the cells of the pericycle which approach maturity (*cf.* fig. 16).

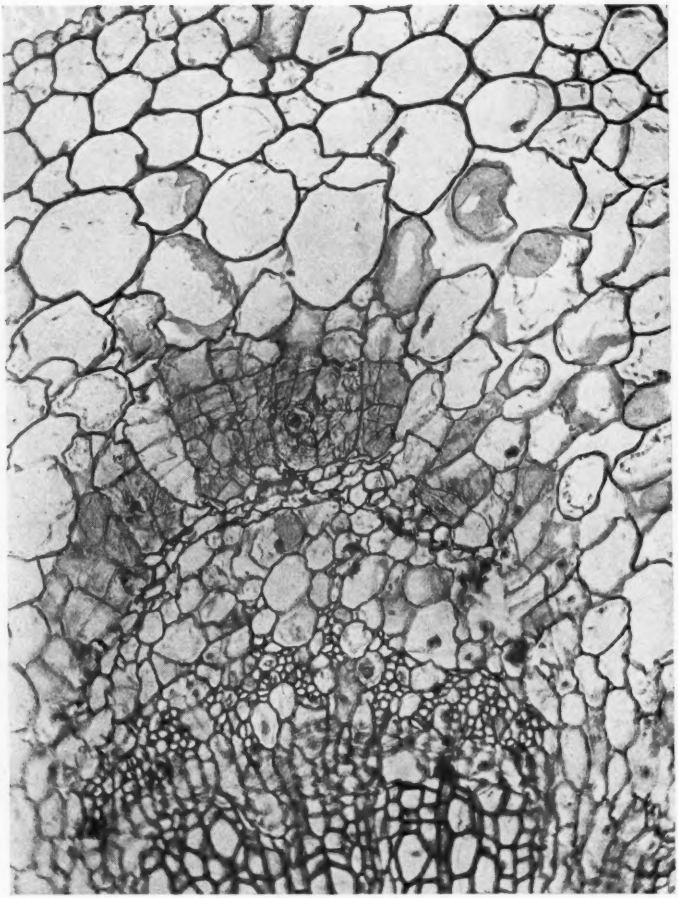

FIG. 22.—Same section as fig. 21 but of another vascular bundle. In this instance a vascular strand is being initiated in the proliferated endodermal cells. Walls of original endodermal cells clearly evident at left. One cell has divided tangentially only, another in several planes (*cf.* fig. 23).

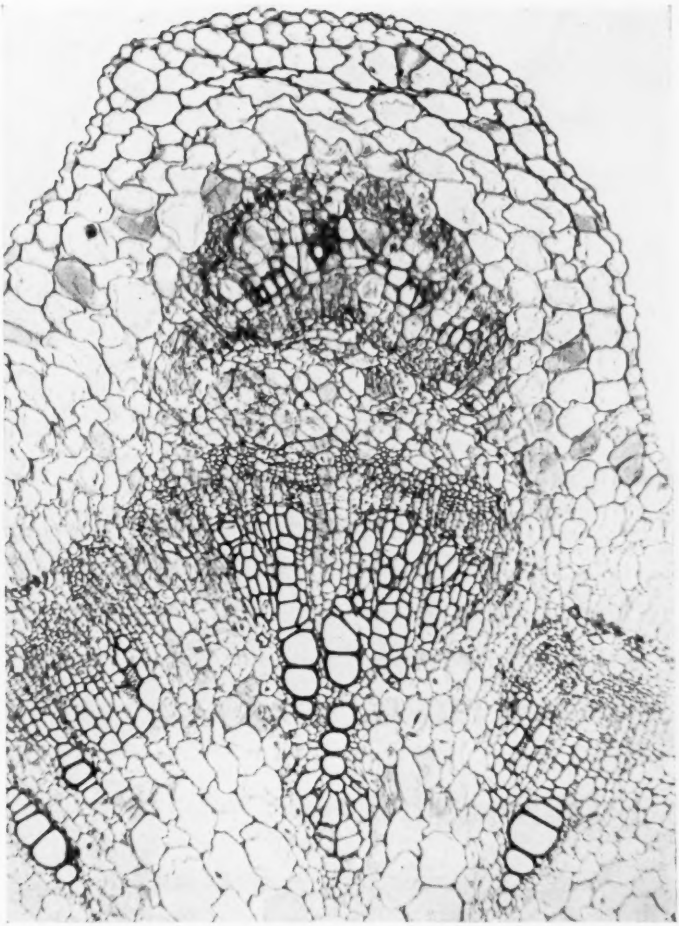

FIG. 23.—Same stem as figs. 21 and 22, section at slightly higher level (3 mm. below treated surface). Vascular elements derived from cells of proliferated endodermis. The whole group is in a more advanced stage than as shown in fig. 22. This section and that shown in figs. 21 and 22 were taken below region in which the apical crown of adventitious roots developed. It is especially noteworthy that in these sections the ray cells show only slight meristematic activity although the pith cells adjacent to protoxylem and metaxylem have proliferated and many derivatives matured as tracheids.

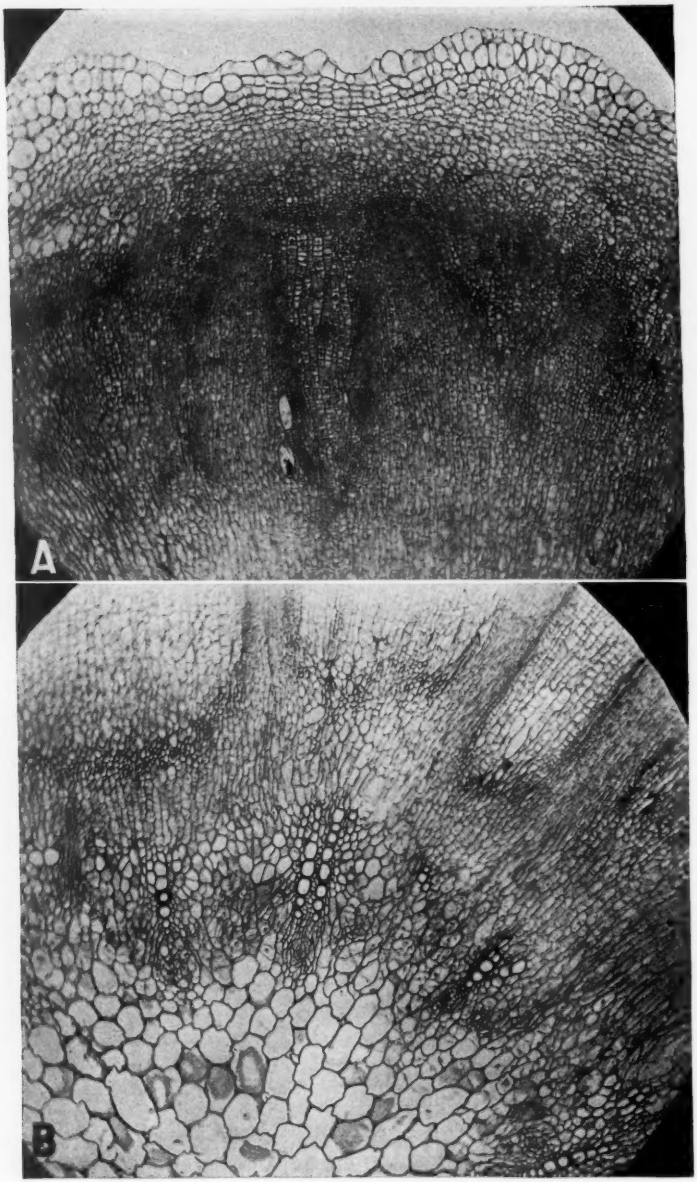

FIG. 24.—*A:* 114 hours after treatment, section 1.2 mm. below treated surface about through middle of zone in which adventitious roots developed, showing two young roots with proliferated endodermal and cortical tissues exterior to them and phloem between and at the sides. *B:* 116 hours after treatment, at approximately same level in stem as *A*. Relation of young roots to phloem, rays, and xylem is obvious.

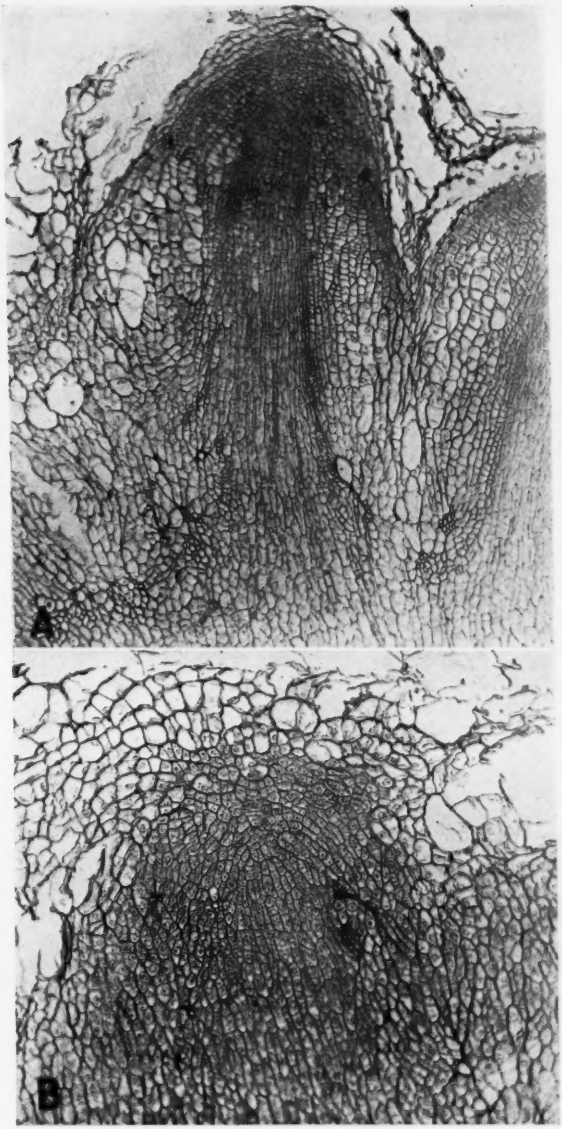

FIG. 25.—*A:* 120 hours after treatment, median longitudinal section of adventitious root. Central core is derived from the ray, its outer portions from the phloem, as indicated by the groups of sieve tubes stretching throughout its length, its tip capped with endodermal and cortical tissue. *B:* 110 hours after treatment, early stage in development of apical histogen. The scattered, denser, irregular patches at either side of central core are sieve tubes of the proliferated phloem.

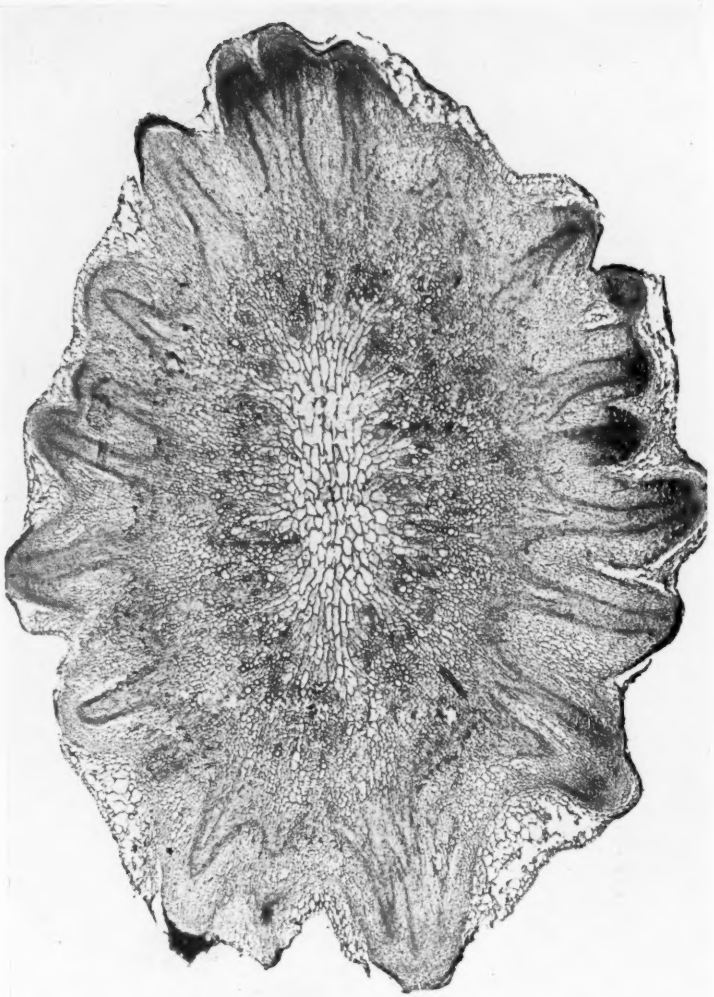

FIG. 26.—One hundred sixty-eight hours after treatment, section about 1.5 mm. below treated surface and through crown of apical roots. The relation of roots to rays and proliferated phloem is shown. Most of the cortical tissues are dead and broken; proliferated endodermal cells are still living and intact at many places over the phloem and over some of the root tips. Pith has proliferated and from the derivatives have come large tracheids, vascular strands, and meristematic zones, bands, and islands (see fig. 28 *A*).

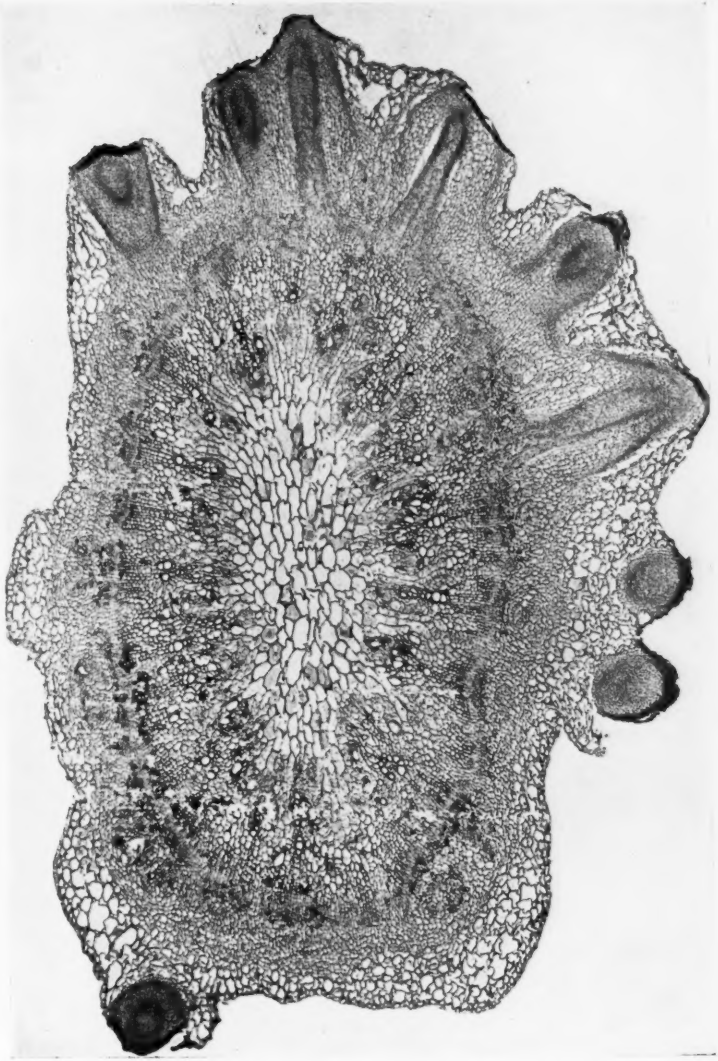

FIG. 27.—Same stem as fig. 26, section at lower level, just below main crown of roots but through several roots at the top. Phloem does not show the extensive proliferation and radial extension that it does in the root zone, nor does the endodermis. Vascular strands have differentiated from the primary phloem in many of the bundles. Rays have proliferated, as has also the pith, and from the derivatives have come vascular strands, tracheids, and masses of meristematic cells.

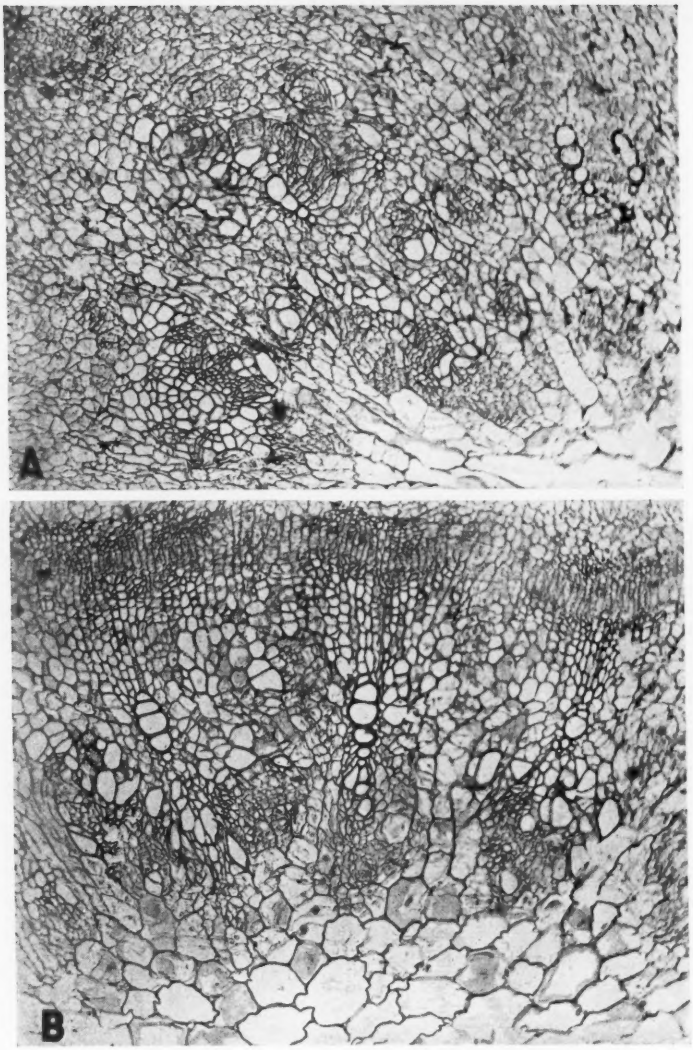

FIG. 28.—*A:* detailed view of portion of rays and pith, as in fig. 26. Following proliferations of the cells of both tissues, a confused mass of vascular elements, parenchymatous cells, and highly meristematic areas has developed. *B:* detail of same regions but at same level as fig. 27. Three points of primary xylem are shown. Outward from them are secondary xylem, cambium, and phloem; inward are large tracheids, vascular strands, meristematic masses, and pith cells showing first divisions and others as yet undivided.

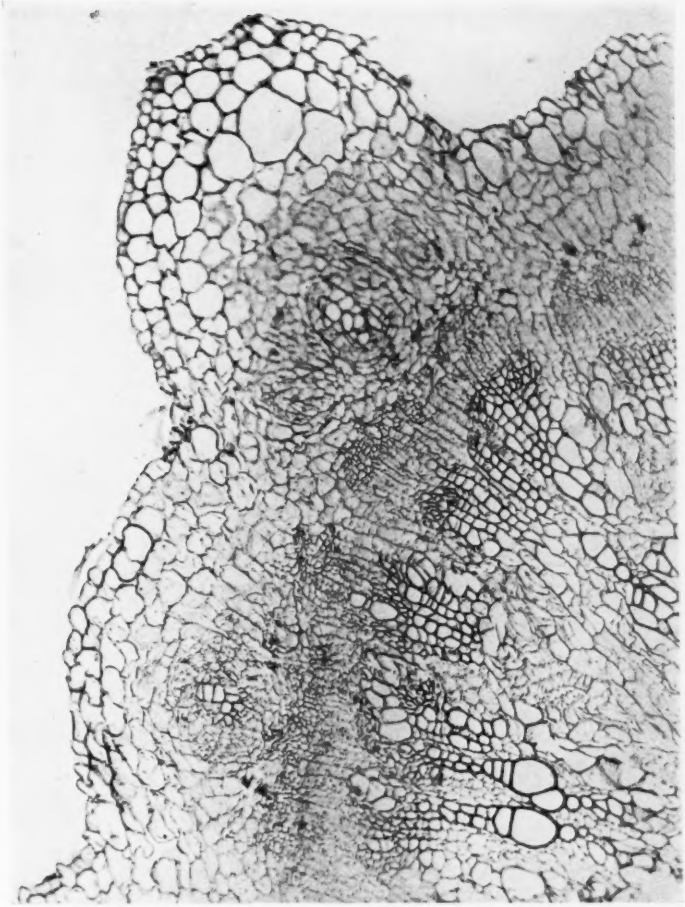

FIG. 29.—Same stem as figs. 26–28, section 6 mm. from treated surface. Vascular strands present in region of primary phloem. These extend for more than 3 cm. down this particular stem. Rays and pith about as in fig. 28.

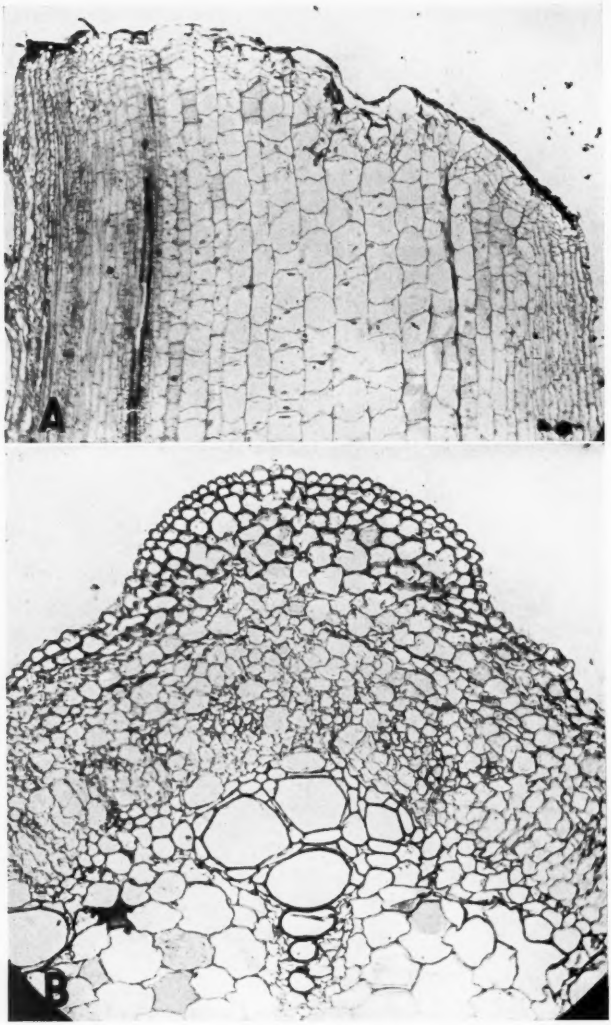

FIG. 30.—*A:* 12 days after treatment of cut surface with pure lanolin only, median longitudinal section of stem. A phellogen has developed across entire stem adjacent to cut surface. *B:* 15 days after treating cut surface with pure lanolin, section 1 mm. below treated surface. None of tissues shows unusual degree of proliferation.

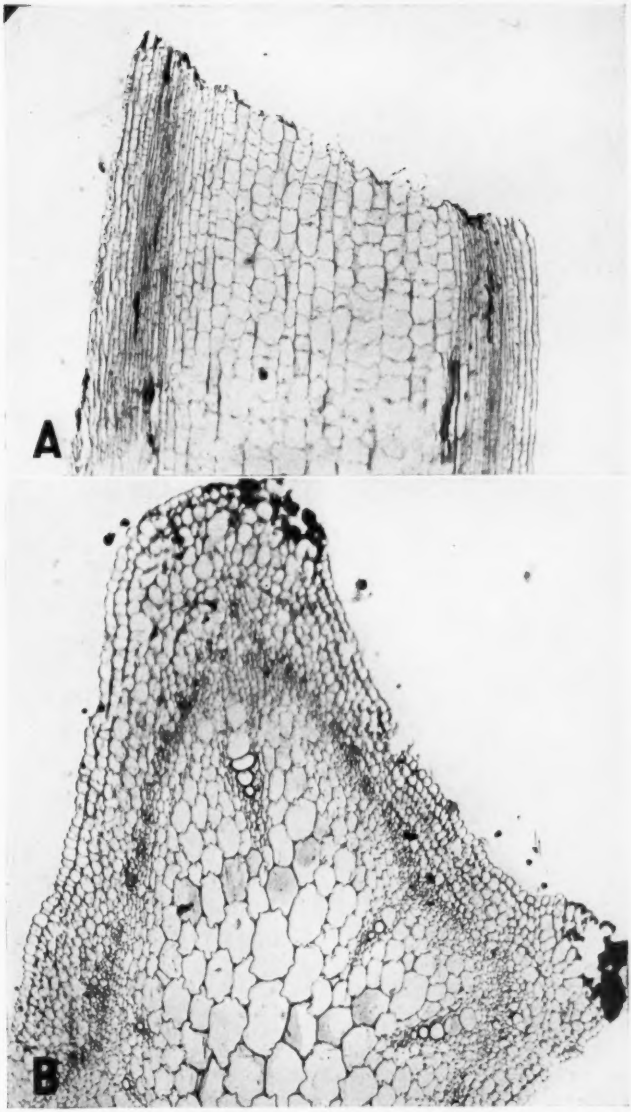

FIG. 31.—Five days after decapitation, the cut surface untreated. *A:* median longitudinal section. *B:* section 1 mm. below cut surface. Cells of some of the tissues are dead, but most of them are alive and show varying degrees of maturation. About half of the stems decapitated and untreated formed no callus and dried out much as those here illustrated; the others formed a callus, as shown in figs. 32 and 33.

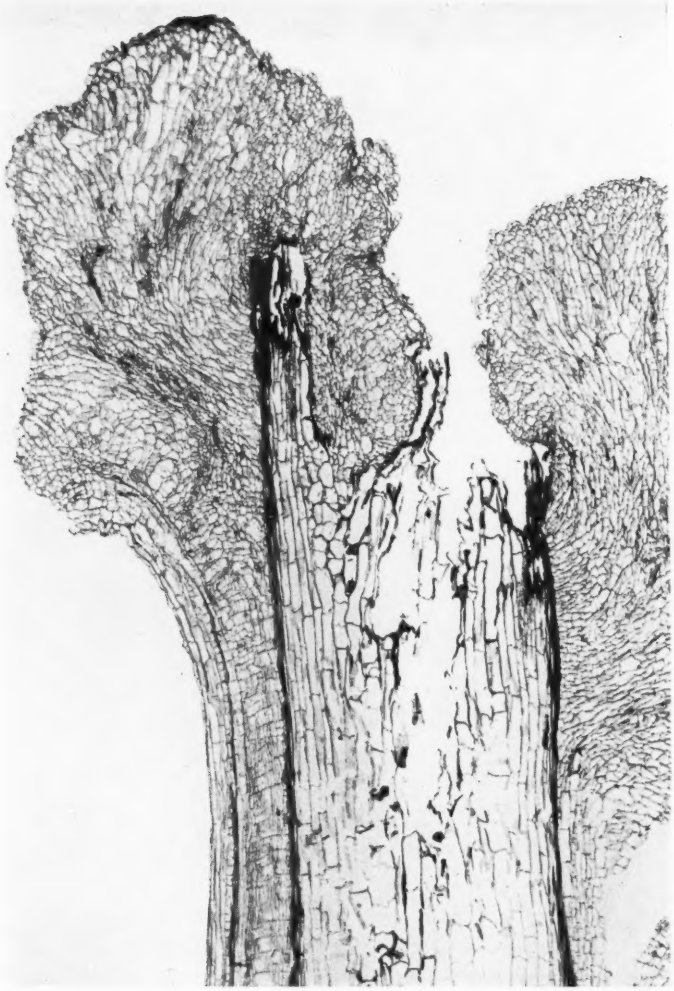

FIG. 32.—Fifteen days after decapitation, the cut surface untreated. Many pith cells are dead and dried out. The callus developed almost entirely from proliferated phloem. At left, below the callus, endodermis is visible as single line of cells three or four cell layers within the epidermis and just outside the outermost black line, the pericycle. Within this is a broad zone of phloem which abuts a narrow band of xylem. In some specimens the endodermal cells at the base of and below the callus show several divisions in the tangential plane.

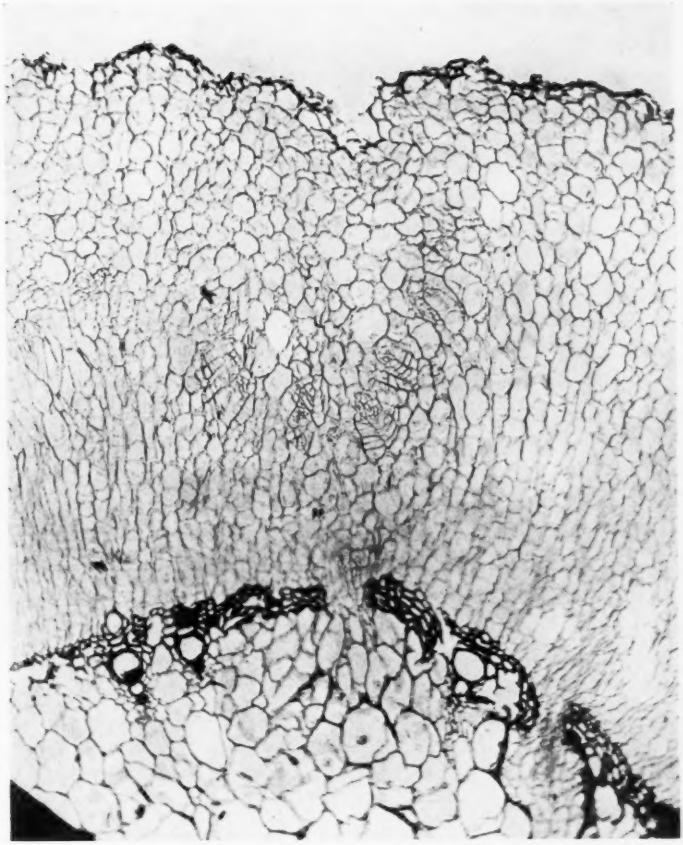

FIG. 33.—Fifteen days after decapitation, the cut surface untreated, section about 1 mm. below original cut surface through callus tissue. Most of the xylem present at time of cutting is dead. A few of the rays, and in places the pith, have proliferated. Most of the callus is from the phloem. Derived cells have matured into tracheids, phloem elements, and large parenchymatous cells. Meristematic activity is general throughout the mass of parenchymatous cells. Outermost cells have suberized.

of the phloem of the original bundles of the stem. A cambium, continuous with the cambium in the adjacent vascular bundles, is differentiated. Apparently it arises from the outermost layer of the inner core of cells derived from the ray rather than the surrounding cells derived from the phloem. New tissues are added from an apical histogen, and as growth proceeds the young root pushes out through the covering layers of endodermal and other cortical cells. It matures in much the same way as do secondary roots.

As has been stated previously, decapitated but otherwise untreated stems generally form a terminal callus over the cut surface, whereas those treated with the lanolin mixture form large irregular tumors. Such calluses are derived mainly through a pronounced proliferation and subsequent partial or complete differentiation of the cells of the phloem. In some instances some of the cells of the ray and of the outer regions of the pith also proliferate. Two specimens examined showed activity on the part of the endodermis, but this is very slight in comparison with the activity manifested by endodermal cells when stimulated with indoleacetic acid. After a time some of the outermost cells of the callus suberize, others mature as large pitted tracheids or phloem elements, some remain as large parenchymatous cells, and many continue actively meristematic. Old calluses show irregularly organized vascular strands scattered through them.

Discussion

In many respects the overgrowths produced on Kidney bean resulting from the application of indoleacetic acid in lanolin resemble the tumors produced by bean and other plants treated with a growth substance extracted from *Bacterium tumefaciens*, as has been pointed out by BROWN and GARDNER (3). SMITH (12), and SMITH, BROWN, and MCCULLOCH (11) have drawn a parallel between the reaction of plant tissues to infections by *B. tumefaciens* and cancerous growth in animals. Many others have also developed a similar viewpoint. In following out this concept, SMITH and his co-workers not only studied the structure and development of tumors resulting from injection of *B. tumefaciens*, but also conducted a number of experiments in which a series of chemical compounds was applied to vari-

ous plant tissues. The cellular and histological responses of the treated plants were studied. While it is not now our purpose to state specifically that the responses of plant tissues to indoleacetic acid are duplicates of those resulting from inoculations with *B. tumefaciens*, yet the similarities are many, as BROWN and GARDNER have indicated. The works of SMITH and his collaborators furnish the most extensive account of the histological details available to us.

The following excerpts from SMITH, BROWN, and MCCULLOCH will give, in part, an indication of their point of view.

"The tumor originates in meristem, usually in the cambium region. It may perish within a few months or continue to grow (parts of it) for years.

"The tumor consists, or may consist, not only of parenchyma cells but also of vessels and fibers, i.e., it is provided with a stroma which develops gradually as the tumor grows. A proliferating tumor usually contains not only meristem but pitted vessels and sieve tubes; it may also contain wood fibers, but does not always.

"The tumor sends out roots (tumor strands) into the normal tissues. These may extend for some distance from the tumor—how far is not known. These strands consist of meristem capable of originating medullary rays, tracheids, and sieve tubes. In the daisy the strand passes through the protoxylem region of the stem. It is rich in chloroplasts. . . . . A considerable part of it consists of unripe, actively vegetating cells.

"In the daisy the infiltrations are not through the vessels, but between them in a tissue offering little resistance to intrusion, i.e., the region occupied by the thin-walled, delicate spiral vessels.

"In the substance of these deep-lying strands secondary tumors develop. These gradually rupture their way to the surface.

"There are no true metastases in crowngall, but this does not, to our mind, militate against the comparison, for whether a cancer shall be propagated by floating islands of tissues, or only by tumor-strands, appears to be a secondary matter depending on the character of the host tissues rather than on the nature of the disease. The essential element is the internal stimulus to cell division.

"The stimulus to tumor development comes from the presence of the parasite within certain of the cells. Apparently it is not in all."

In brief, SMITH (12) assumed that *B. tumefaciens* lived mainly as an intracellular parasite, but the immediate or proximate cause of the phenomena of cell proliferation "must be the chemical or physical action of enzymes or other substances produced or activated by the bacteria as a result of their metabolism, with a corresponding *reaction* on the part of the plant." Some of the cells of the host might show definite reactions to the foreign organism although they were not in contact with the bacterial cells and such response might be situated at some considerable distance from them.

RIKER (10), BANFIELD (2), and others have suggested that the bacterial organism concerned in the development of crowngall is intercellular. According to BANFIELD, who worked with cane gall, the "cells with which the bacteria are in contact undergo a more or less rapid cytolysis. In early stages of gall formation those cells at a distance from the bacteria divide rapidly. Subsequently intercellular penetration may occur, and in turn those cells at a distance may be incited to extensive division. Eventually cell division ceases, extensive intercellular penetration by the bacteria continues, and the gall degenerates."

Each hypothesis, whether it postulates the foreign organism as intracellular or intercellular, includes the suggestion that the living cells of the host react to some substance generated by the bacteria, and that the bacteria need not be in direct contact with the cells which have been stimulated to divide.

The histological responses of the cells of the bean plant to indoleacetic acid show many developmental patterns which are almost exact duplicates of those SMITH, BROWN, and MCCULLOCH have shown for crowngall. Those which follow the application of the indoleacetic acid are somewhat more pronounced. It is not entirely clear whether SMITH considered the tumor strands of crowngall as strands of cells which interpenetrated other tissues and then enlarged and differentiated subsequently or whether strands of cells *in situ* were stimulated, became meristematic and their derivatives differentiated into the many and varied forms he illustrated. Certain it is that his photomicrographs clearly indicate the latter to have been the case. We have obtained such changes by the use of indoleacetic acid, the resultant structures having the same appearance as

tumor strands. The prolonged meristematic activity of some of the tissues and the continued differentiation of aberrant structures from some of the regions, such as the pith, following treatment with indoleacetic acid, warrant much further experimentation and study. Whether such behavior on the part of the tissues is to be likened to tumor growths in animals must await subsequent findings.

What, in cytological and physiological detail, may be the character of the response of cells to stimulation by growth substances awaits further investigation. JONES (**7, 8, 9**) has recently suggested that there is a "possibility that missing genes due to corresponding deficiencies in certain parts of both members of a chromosome pair result in a chromosomal unbalance and this brings about unregulated growth. . . . . The well-known effect of x-rays, radium, hormones, and other powerful physical and chemical agents in bringing about unregulated growths seems conclusively to be due in some way to their action upon the chromosomes resulting in the loss of parts or of whole chromosomes." SMITH, BROWN, and MCCULLOCH showed that in the development of crowngall there are many and varied amitotic and mitotic divisions of the nuclei. In our present work we have not yet carried out cytological investigations. Such investigations would be of great value, although it does not seem necessary to assume that there must be chromosomal changes or any type of mutation to account for the histological developments observed. As has been pointed out, the several main tissue systems differ widely in their degree and type of response to stimulation. As far as could be determined from the studies made, none of the types of cells developed was fundamentally different from cells occurring in bean plants grown under the usual conditions of culture and environment, although the sizes, numbers, and relative proportions of the various types of cells show wide departures from the conditions found in such plants. One of the most striking responses following the application of the lanolin mixture is the tendency of cells to become highly meristematic and for some of the derived tissues to remain in such condition for long periods of time. Further details in this connection will be given when tumors older than 168 hours are discussed.

Among other responses, the pronounced development of vascular

strands from the tissues of the endodermis, primary phloem, pith, and other regions, which under the general cultural conditions of the bean are not generally encountered, emphasizes again the degree to which differentiated cells retain their totipotential capacities.

In discussing crowngall, SMITH summarizes some of his concepts on growth as follows: "Growth is the normal function of cells. They are always multiplying when they are not inhibited by one thing or another. Growth, then, if this view is correct, comes about not by the direct application of stimuli, but indirectly *by the removal of various inhibitions*. Under normal conditions the physiological brakes are on at all times, more or less, in both plants and animals, and only when they are entirely or largely removed in particular areas do we observe an unlimited cell proliferation resulting in the hasty and peculiar growths known as neoplasms or cancers. . . . . The inhibition remover we are in search of is *one that acts locally, disturbing tissue equilibriums within limited areas*." SMITH has considerably more to say concerning the relative importance of chemical and physical factors as stimuli or as removers of inhibition. Any critical evaluation of the various views advanced concerning the phenomenon of growth, the rather vague term unregulated growth, and the like, are beyond the scope of the present paper.

Perhaps it will be possible eventually to approach somewhat more closely an explanation of some growth responses of cells through the use of an increasing list of growth substances available as chemically pure compounds. But as yet, even though it may be possible to account for the behavior of cells, it is not possible to account physiologically for the developmental patterns of tissues, largely because so few detailed patterns related to specific stimuli are available for critical examination.

Summary

1. The living cells of the stem of Red Kidney bean are responsive to applications of indoleacetic acid in lanolin. The mixture used consisted of 30 mg. of indoleacetic acid per gram of pure lanolin.

2. Young bean seedlings were decapitated by cutting the stem off squarely at the top of the second internode of the primary axis. The

cut surface was left untreated or smeared once with a thin coating of pure lanolin or the lanolin-indoleacetic acid mixture.

3. Gross observations extending over several weeks were made on the untreated and treated stems without removing them from the plant. Histological studies were made of material collected and preserved at 6-hour intervals up to a total of 168 hours. The several tissues responded variously to treatment, depending in part upon their distance from the surface of application of the mixture.

4. The epidermal cells undergo a few divisions in the radial plane and enlarge somewhat after treatment.

5. The cells of the cortical parenchyma enlarge somewhat and those near the endodermis become meristematic. Starch grains and chloroplasts disappear from them when activity is initiated. After a variable number of hours the outermost cells die and in the vicinity of the root tips they are pushed aside and disrupted. At distances more than 5 mm. from the surface of application the cortical cells show little histological change but may lose their content of chlorophyll and starch for a time; at later stages these again appear.

6. The cells of the endodermis are highly responsive to indoleacetic acid. Nuclear division is greatly speeded up shortly after application. Walls subsequently form in all planes, especially the tangential. Within 168 hours cells 5 cm. below the point of application are highly meristematic. The derived cells differentiate as xylem and phloem elements and large, often multinucleate, parenchymatous cells. Many remain meristematic and still others give rise to adventitious roots. Especially over the vascular bundles, long proliferating strands of vascular tissue are developed. These frequently enlarge to the extent that they rupture the tissues exterior to them and appear as tumors in rows along the stem.

7. The cells of the pericycle proliferate slightly and mature as parenchymatous cells, or, if some distance from the lanolin mixture, as thick walled blunt fibers.

8. The parenchyma of primary phloem shows the same general type of response and subsequent differentiation of tissues as does the endodermis. In addition some of the cells derived from it form a part of the outermost tissues of the apical crown of adventitious roots.

9. The parenchyma of the secondary phloem undergoes marked proliferation. Most of the tissues composing the outer portion of the apical crown of adventitious roots are derived from it. Other cells mature into parenchymatous tissue, tracheids with simple pits, sieve tubes, and companion cells.

10. When stems are decapitated and otherwise untreated, calluses may be formed. These are derived mainly from the proliferation of the parenchymatous cells of the primary and secondary phloem parenchyma.

11. Decapitated stems treated with lanolin only, thereafter generally enlarge but slightly. The tissues mature and a weak phellogen is formed just beneath the cut surface. Numerous chloroplasts develop in the cortical cells which also contain large deposits of starch.

12. The cambium divides actively; its derivatives may continue their meristematic activity over a considerable period of time, later maturing as various phloem and xylem elements or continuing as meristematic islands or zones from which such elements continue to be derived.

13. Near the surface of application the cells of the ray adjacent to the xylem proliferate greatly. Many of their derivatives mature as tracheids, and commonly a confused system of vascular strands intermingled with highly meristematic areas is developed from them. This confused mass of tissues may persist and continue development for many weeks. The cells adjacent to the phloem and just within the pericycle also proliferate greatly, elongate radially, and in conjunction with the phloem cells flanking them form the principal portions of the apical crown of adventitious roots. The histogens of such roots seem to originate mainly in cells derived from the rays. This is true also for the cambium and xylem of the adventitious roots.

14. The pith cells proliferate greatly. Activity is first noticeable directly adjacent to the surface of application. As time goes on activity progresses down the stem next to the elements of the protoxylem and metaxylem. At still later stages activity may extend to the center of the stem. Maturation of the derived cells results in large pitted tracheids adjacent to the elements of the primary xylem and the formation of a confused mass of vascular strands, scattered

vascular elements, large parenchymatous cells, and meristematic zones and islands where the pith formerly was. These tissues continue to develop for a long period of time and account for most of the overgrowths which come to overtop the apical tumor and eventually attain a large size.

15. The histological developments following the application of indoleacetic acid closely resemble many of those associated with crowngall produced by *Bacterium tumefaciens*.

16. The most striking feature shown by stems treated with lanolin mixture is the very great speeding up of nuclear divisions, especially in cells near the surface of application. Such divisions result in a multinucleate condition of many of the cells. Later cell division occurs and the derived cells may remain highly meristematic for long periods of time.

17. It does not seem essential to assume that the developmental patterns expressed by tissues following treatment necessarily result from an effect altering the genetic composition of the chromosomes to the degree that parts of chromosomes or whole chromosomes have been lost.

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IS PROTOPLASM ELASTIC?[1]

HENRY T. NORTHEN

(WITH ONE FIGURE)

A controversy exists as to whether protoplasm behaves like a true fluid (1) or whether it behaves like an anomalous fluid (2). If protoplasm is a true fluid it should possess one viscosity value; that is, the value should be independent of the pressure or shearing stress. On the other hand, if protoplasm is an anomalous fluid it will possess an infinite number of viscosity values, the value obtained depending upon the pressure or shearing stress.

According to SEIFRIZ (2), fluids which possess structural aggregates are elastic. Hence if it can be demonstrated with certainty that protoplasm is elastic, it can be inferred that protoplasm is built up of intermeshed, linear, crystalline units. The following data demonstrate that the "viscosity" of cytoplasm of *Zygnema* sp. varies with the shearing force and is therefore elastic.

The method used was based on Stoke's law which governs the rate of movement of a particle through a fluid. According to this law:

$$L = 2cg(D - d)a^2t/9\eta \qquad (1)$$

in which L is the distance moved in the time t, g the gravity constant, (D − d) the difference in specific gravity between the moving particle and the surrounding medium, a the radius of the particle, η the viscosity of the medium, and c a constant which may be determined from the following equation:

$$c = 4.024rn^2 \qquad (2)$$

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 159.

in which r is the radius of the centrifuge in meters and n the number of revolutions per second. From equation (1): $L \propto ct$, and $\eta \propto 1/L$. Hence $\eta \propto 1/ct$. From equation (2): $c \propto n^2$. Hence $\eta \propto 1/tn^2$. From a consideration of the last proportion: if protoplasm is a true fluid, the same value of η should be obtained as long as the product of tn^2 remains constant. If protoplasm is elastic, η should

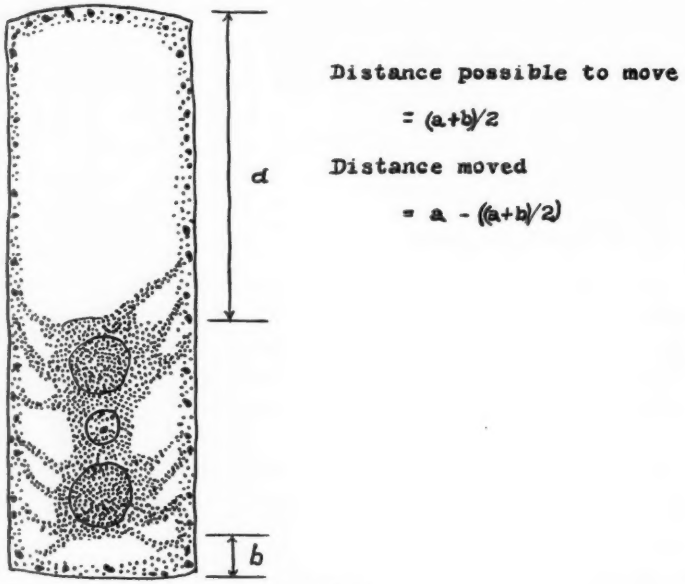

FIG. 1

be lower when n is small and t large even though the product of tn^2 remains constant.

In the following experiments, filaments of *Zygnema* sp. were centrifuged at different speeds with a hand driven centrifuge, but for any one experiment the product of tn^2 was constant. Following centrifugation the filaments were mounted in a 1 per cent solution of eosin which likewise contained 1 cc. of acetic acid per 100 cc. of solution. It was observed that mounting in this solution maintained the chloroplasts for hours in the same position as they were a moment after centrifugation.

The distances the chloroplasts move in response to centrifugal force were determined as shown in figure 1, and the results are given in table I.

In experiment number one, the filaments were centrifuged for too long a period to bring out any significant difference between the cells centrifuged with a force of 471.9 × gravity and those centrifuged with a force of 118.0 × gravity.

TABLE I

MOVEMENT OF CHLOROPLASTS IN RESPONSE TO DIFFERENT SHEARING FORCES

EXPERIMENT NO.	NO. OF CELLS MEASURED	c FORCE × GRAVITY	SECONDS CENTRIFUGED	TC	DISTANCE POSSIBLE*	DISTANCE MOVED* (FLUIDITY)	RELATIVE VISCOSITY (1/FLUIDITY)
1	54	471.9	90	42471	0.89	0.86	1.16
	63	118.0	360	42471	0.88	0.869	1.15
	72	29.5	1440	42471	0.811	0.722	1.38
	57	13.6	3120	42471	0.781	0.612	1.63
2	84	118.0	240	28320	0.895	0.602	1.66
	97	29.6	960	28320	0.793	0.287	3.48
	119	7.4	3840	28320	0.807	0.273	3.66
3	51	118.0	240	28320	0.80	0.49	2.04
	54	29.6	960	28320	0.711	0.209	4.78
4	54	118.0	240	28320	1.023	0.946	1.06
	93	29.6	960	28320	0.895	0.535	1.87
	111	7.4	3840	28320	0.944	0.362	2.76
5	83	679.9	30	20397	0.896	0.724	1.38
	101	170.0	120	20397	0.882	0.608	1.64

* In relative units.

It might be expected that the corresponding viscosity values in experiments 2, 3, and 4 would agree more closely. For example, in experiment 2 a value of 1.66 is recorded for the cells centrifuged with a force of 118.0 × gravity whilst in experiment 4 a value of 1.06 is recorded for those centrifuged with a similar force. There are several reasons for these discrepancies. First, the cells vary in length, and in general the longer the cell, the more readily will the chloroplast move. The column "Distance possible" gives an indication of the average length of the cells which were measured. Second, the physio-

logical state of the cell at the time of experimentation must be considered. The rate with which the chloroplast moves through the cytoplasm will vary from morning to afternoon, from afternoon to evening, and from day to day. The "viscosity" of the cytoplasm will vary with time, but perhaps more important is the change in the relative specific gravity of the chloroplast, which will depend upon the amount of starch stored within and immediately around the chloroplast. To eliminate as far as possible the variations due to time, the centrifuging in each experiment was conducted within a period which did not last over two hours.

From a consideration of the relative "viscosity" values obtained in this experiment, the following conclusions are justified: First, no single viscosity value can be ascribed to the cytoplasm of *Zygnema* cells. There are an infinite number of viscosity values and the value obtained will depend upon the shearing force—the greater the force used, the lower the apparent viscosity. Second, the cytoplasm of *Zygnema* cells is elastic.

Summary

1. Filaments of *Zygnema* sp. were centrifuged with different forces, but in each experiment the product of time centrifuged and the square of the number of revolutions per second remained constant.

2. The experiments demonstrate that no single viscosity value can be ascribed to the cytoplasm of *Zygnema* cells. The value obtained depends upon the shearing stress—the lower the stress, the higher the apparent viscosity.

3. It can be concluded that the cytoplasm of *Zygnema* cells is elastic.

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OFFSPRING OF A SELF-POLLINATED REVERSED CARPELLATE PLANT OF MORUS ALBA[1]

JOHN H. SCHAFFNER

In 1929, the writer published the results of a breeding experiment with self-pollinated seeds obtained from a staminate plant of *Morus alba* L. which had several branches showing sex reversal to femaleness (2). The progeny from these seeds consisted of pure staminate individuals, pure carpellate individuals, and individuals with mixed sex expression at the first blooming period. It was evident, therefore, that in the white mulberry an individual originally determined as a pure male had the potentialities to produce offspring of both sexes without the introduction of any new hereditary units from the outside. Sex reversal had been seen previously in carpellate plants but no trees were available at this time for experiment, although young trees were being grown in order to make a definite study of reversal to maleness in carpellate individuals. These young trees were transplanted from the greenhouse to the botanic garden, and in 1932 a carpellate plant showed several small branches with reversal to maleness, developing a few staminate catkins. These branches were inclosed with some carpellate branches with cloth to keep out all foreign pollen, and a small quantity of selfed seeds was obtained. These seeds were dried for a few days and then planted in the greenhouse. The seedlings developed vigorously in a long-light photoperiod during the winter, and the next spring (1933) they were planted in rich soil in the garden. In the meantime, the spring of 1934 was very dry and the original parent tree, now ten years old, bloomed abundantly and showed a decided increase in the number of reversed branches and the number of staminate catkins and flowers. Nearly all of the main branches of the tree now developed some staminate catkins. There was a decided gain in maleness in this tree since the spring of 1932. In the regions of sex reversal many of the catkins were poorly developed, with part of the flowers either vestigial or distorted. In some cases ovularies were found with imperfect anthers developing in their sides.

[1] Papers from the Department of Botany, The Ohio State University, no. 379.

Of the plants obtained from the selfed seeds twenty-four survived, and in the spring of 1935 two trees had attained reproductive maturity and each produced a few pure carpellate catkins. None of the other trees developed any flowers and several of them were still quite small, because of several years of unfavorable conditions.

In the spring of 1936, most of the trees were from 6 to 12 feet high, and by May 8 twenty of them were in bloom, while the smallest trees still developed no flowers. Of the twenty trees that bloomed, five were pure staminate in sex expression, eight were pure carpellate, four were decidedly staminate but produced some carpellate flowers, one was decidedly carpellate but had several catkins with some staminate flowers, and two were of decidedly mixed sexual expression, mostly with catkins showing both carpellate and staminate flowers. The eighteen trees which were either pure carpellate or pure staminate in sex expression or very decidedly female or male thus showed an equal number for each sex, nine female and nine male. Although there were only five pure staminate trees to eight pure carpellate, the difference is not significant because of the small numbers; but the general results are significant, since they show that in *Morus alba* an hereditary constitution, which was originally determined as female, can without any addition from outside sources produce offspring which at their first blooming period are pure male and pure female, just as an hereditary constitution which was originally determined as male can produce both pure male and pure female offspring without any additional heredity from outside sources. The two trees which were pure female at their first blooming period in 1935 continued as pure females in 1936 and produced an abundant crop of fruit.

It is evident that no hypothesis based on the notion of a homozygous-heterozygous sex-determining mechanism of Mendelian sex genes or factors will explain these results. Both the individuals which are originally determined as pure male and pure female contain the potentialities for both sexes, and the determination of the one sex or the other is then necessarily dependent on a differential physiological state brought about through some physico-chemical condition present at the time in the cells concerned, whether it be the original determination in the egg or a later determination or sex reversal in the vegetative buds or tissues. In dioecious plants the orig-

inal sex determination probably mostly takes place by a swing of the physiological sex balance, in the egg, either before fertilization, at the time of fertilization or the formation of the zygote, or immediately after the fertilization process has been completed. In some special cases, however, as for example in *Arisaema triphyllum* (1), it appears that the sex balance is originally always in the direction of maleness, the young plants all being staminate, while in the mature condition the individuals are pure staminate and pure carpellate. It is established through experimental means that the mature individuals of *A. triphyllum* are balanced equally in respect to sex determination and sex constancy, the reversal from one sex to the other being induced as easily in one direction as the other.

It is evident that it is impossible to determine the sex reactions of a species by observations on only a single reproductive period, whether the first or any subsequent one. To determine the true sexual nature of the individual or race, all available extreme ecological environments must be tried before a formula can be established, either for the sexual constitution or for the ordinary Mendelian gene constitution concerned in the determination of secondary sexual characters. *Morus alba* evidently contains a complete complement for both sexes in every individual. The stability or instability of differentiated systems is not a condition peculiar to sexual phenomena but is in evidence in the tissues and organs in both the higher plants and animals. In some cases remarkably stable systems are differentiated from the heredity complex while in others the differentiated tissues may be dedifferentiated and a new differentiation process initiated without adding to or subtracting from the complex of hereditary units. These reversals and dedifferentiations, which play such a prominent part in sexual phenomena, indicate that the sex determinations are caused by physiological or physico-chemical states in the protoplasm and not by hereditary differentials through the shifting of chromosomes or other protoplasmic organs; and from this point of view, the results of the experiments with *Morus alba* become intelligible.

It is evident that if the physiological view of sex determination is correct, different species, having distinctive, general, hereditary constitutions, may give quite different results from reversed selfed plants. The following six types or categories of simple sex expression are the-

oretically possible: (1) both males and females may produce both sexes, as is the case in *Morus alba;* (2) males may produce only males and females only females as YAMPOLSKY found in *Mercurialis annua;* (3) males may produce both sexes while females produce only females; (4) females may produce both sexes while males produce only males; (5) both reversed males and females (as well as ordinary cross pollinations) may produce offspring all of which are originally determined as males, as appears to be the case in *Arisaema triphyllum;* (6) both reversed males and females may produce offspring all of which are originally determined as females.

Summary

1. A reversed carpellate tree of *Morus alba* was self-pollinated and from the seed produced twenty-four young trees were grown. At the first blooming period only two trees bloomed and these were pure carpellate. At the second blooming period, a year later, twenty of the twenty-four trees bloomed and of these five were pure staminate; eight (including the two carpellate trees of the previous season) were pure carpellate; four were decidedly staminate but each one had some carpellate flowers; one was decidedly carpellate but had several catkins with some staminate flowers; and two were of decidedly mixed sex expression.

2. These results correspond with those obtained several years earlier from a reversed staminate tree from whose self-pollinated seed a progeny was developed also consisting of pure males, pure females, and individuals of mixed sex expression. The results from the two experiments show that the dioeciousness or unisexuality of *Morus* is not caused by hereditary differentials, like an "xy" or "wz" allosome set or by any of the newer factor hypotheses, but by a physico-chemical or physiological condition, since both male and female individuals are potentially bisexual. This conclusion is confirmed by the frequent sex reversals which take place in both young and old trees.

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CURRENT LITERATURE

Growth Hormones in Plants. Authorized English translation of *Die Wuchsstofftheorie und ihre Bedeutung für die Analyse des Wachstums und der Wachstumsbewegungen der Pflanzen.* By P. BOYSEN JENSEN. Translated and revised by GEORGE S. AVERY, JR. and PAUL R. BURKHOLDER, with the collaboration of HARRIET B. CREIGHTON and BEATRICE A. SCHEER. McGraw-Hill Book Co., New York. 1936.

The German text by P. BOYSEN JENSEN, which appeared in 1935, attempted to present the chief lines of investigation and the main results of research done in the last 25 years. The views of various investigators were presented objectively, and experimentally founded facts and hypothetical views were distinguished. Particular attention was given to the fundamentals of the growth substance theory.

The new book is more than a translation and revision, as those terms usually indicate. It is essentially a new text which takes advantage of all the information in the German text and adds results of investigations to date from all over the world. There are 40 new figures and illustrations, and approximately 200 new citations. No attempt has been made to present a literal translation of the German edition. The reader will frequently wonder whether the versions belong to JENSEN or to the American authors. To the student this will make little difference; to the scientist, it may.

The book has been reorganized, certain chapters rearranged, and parts condensed or expanded. The final chapter of the German volume is broken up and distributed through earlier chapters. New features are a summary at the end of each chapter, a substantial index, and historical reviews presented with a series of diagrams.

The historical review will help students to understand how subject matter is developed through years of effort. Sixty-four illustrations enrich the volume and make it more understandable by those less familiar with this new subject. The book is literally filled with references, going back to the time of SACHS and DARWIN. The real starting place, however, is the published work of BOYSEN JENSEN in 1910, with conclusions based on experimentally founded facts. Using the coleoptile of *Avena*, decapitated and otherwise, BOYSEN JENSEN showed that the conduction of the stimulus causing phototropic curvature takes place on the shaded side by the downward movement of a growth-promoting substance. Since that time, great advances in our knowledge have been made, but the book makes clear that there are still many points needing further investigation. Scientists throughout America will certainly welcome this English text.—P. W. ZIMMERMAN.

Die Süsswasser-flora Mitteleuropas, Heft 15: Pteridophyten und Phanerogamen, unter gleichzeitiger Berücksichtigung der wichtigsten Wasser- und Sumpfgewächse des ganzen Kontinents von Europa. By H. GLÜCK. Jena: Gustav Fischer, 1936. Pp. xx+486. Figs. 258.

During the past three decades the researches of H. GLÜCK upon the life histories and ecological relationships of the European Alismaceae and various species of *Utricularia*, also his foliar and floral studies of various other aquatics and near-aquatics, have received an important place in botanical literature. The present work is the fifteenth and numerically the final volume in the series of brilliant treatises on the fresh-water flora of central Europe being issued and in part written by Dr. ADOLPH PASCHER of Prague. (Certain volumes dealing with the simpler plants are still in process of completion.)

Unlike the fungi and several other groups of lower plants which have numerous species that are more or less cosmopolitan, the plants of the present text mostly display pronounced restrictions of range. Thus the European plants included by GLÜCK (his treatment is not limited to central European plants) are largely absent from North America. His descriptions and illustrations will prove interesting to American botanists, however, because of the numerous comparable or analogous habitat forms to be found in our own flora. The text abounds, as do some of GLÜCK'S earlier writings, in *formae*, varieties, and hybrids. A *forma* with GLÜCK is often what American taxonomists would designate merely as a "state." To a local (central European) ecologist, the inclusion of every discernible habitat state as a *forma* or even *subforma* may seem desirable, if only as a teaching aid. For taxonomists, however, the dignifying of innumerable local "states" with names implying formal taxonomic status may well inspire misgivings.

The volume is small and will fit many pockets. The figures are simply drawn but exceedingly clear and helpful. Unfortunately, the paper used is of an impermanent type such as is all too common in scientific books of the present day. It is much to be desired that works of major scientific value, such as GLÜCK'S really is, may be issued, even if in a small supplementary edition, in a permanent form for libraries.—E. E. SHERFF.

Monographie du Genre Cestrum L., part I. By PIERRE FRANCEY. Candollea 6:46–398. 1935.

In a work which won the DECANDOLLE prize in 1934, FRANCEY offers the first part of a monographic arrangement of the Solanaceous genus, *Cestrum* L.

The treatment opens with a preface and this is followed by two parts, general and systematic. The former presents a historical view of *Cestrum*, distinguishes it from the allied genus *Sessea*, discusses its morphology, and gives in detail an account of the geographical distribution, which is almost exclusively American. The systematic section, which is of course the main part, contains a total of 205 species, the first 28 being placed in Section Habrothamnus

(Endl.) Schlecht. and the rest in Section Eucestrum Dunal. The descriptions, which are especially full, are in Latin throughout, as are also the keys, while the supplementary text is in French. Types are not specially indicated, and in the case of some species this is apt to prove vexing. In general, however, high standards of excellence are uniformly maintained. Among monographs which have appeared during the present decade, FRANCEY's will easily rank as one of the very best.—E. E. SHERFF.

Experimental Enzyme Chemistry. By HENRY TAUBER, Burgess Publishing Co., Minneapolis, 1936. Pp. 118. $3.50.

The rapid advances made in recent years in the experimental study of enzyme chemistry are summarized briefly in a mimeoprint book by Dr. HENRY TAUBER, of the New York Homeopathic Medical College and Flower Hospital, New York. No attempt is made to present the more theoretical aspects of the subject; the author has tried chiefly to summarize the recent progress in methods and results of experimental studies in the field of enzyme reactions. The introductory chapter includes brief reference to many subjects, such as temperature effects, pH, specificity, mass action, development of equations, inhibition, inactivation, kinetics, antiseptics, mechanism of enzyme reactions, enzyme syntheses, and the preparation of enzyme materials.

The succeeding chapters are devoted to groups of enzymes: esterases, proteolytic enzymes and peptidases, amidases, carbohydrases, catalase, oxidizing enzymes, the flavin oxidation system, carbonic anhydrase, the zymase complex, and luciferase. The material is usually presented in brief paragraphs that state what has been done, with citation of the papers containing the original results. Extensive bibliographies accompany each chapter.

Although it contains but 118 pages, this volume is condensed and contains considerable information. For those who wish a summary of recent experimental techniques and results, it will be a helpful guide. It does not take the place of the original literature, but it does cite much of it, so that one may quickly become familiar with the most important recent advances in any part of the field.—C. A. SHULL.

Statistical Methods for Research Workers. By R. A. FISHER. 6th ed. Oliver & Boyd, London, 1936. Pp. xiii+339.

Statistical Methods in Biology, Medicine and Psychology. By C. B. DAVENPORT and MERLE P. EKAS. John Wiley & Sons, New York, 1936. Pp. xii+216.

Those who desire to study their problems by statistical methods have a choice of two excellent manuals offered to them in new editions. The first is the sixth edition of FISHER's statistical methods for research workers; the second is the fourth edition of the work by DAVENPORT and EKAS. Both of these works have been long and favorably known, and description is superfluous. Only a few changes are noted in FISHER's new edition, which was also true of all

earlier editions. Section 22, ex. 15.1, presents a complex test on homogeneity in data with hierarchical subdivisions; section 26 makes reference to WORKING and HOTELLING'S formula for sampling error of values estimated by regression; and section 29.2 directs attention to an extended use of successive summation in fitting polynomials. Table 2 has also been extended to the 0.1 per cent level of significance. Earlier editions need not be discarded for these few additions.

The work by DAVENPORT and EKAS has been thoroughly revised, FISHER'S methods for small samples being incorporated into it, but it still retains the general character of former editions. Either manual provides excellent guidance into the statistical analysis of observations made on large or small numbers of individuals or groups.—C. A. SHULL.